Conservation outcomes arising from research into the population genetics, taxonomy and reproductive ecology of the endangered plant *Zieria prostrata*

By

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A thesis submitted for the degree of Doctor of Philosophy
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July 2001

Declaration

I hereby declare that this thesis is my own work and contains no material which has been accepted for the award of any other degree or diploma in any university, and to the best of my knowledge, contains no material previously published or written by any other person except where due reference is made in the text of the thesis.

Patricia May Hogbin

Publications arising from research

Listed below are co-authored publications arising from my research while studying for the degree of Doctor of Philosophy at the Australian National University. Some components of this thesis, although being based in part on these publications, include additional analyses and interpretation. A copy of each publication is included at the back of this thesis.

Hogbin, P. M. and Peakall, R. (1999). A critical evaluation of the contribution of genetic research to the management of the endangered plant *Zieria prostrata*. *Conservation Biology* **13**, 514-522.

Hogbin, P. M. and Peakall, R. (2000). The effective management of threatened flora: lessons from the case of *Zieria prostrata*. *Pacific Conservation Biology* **6**, 238-44.

Hogbin, P. M., Peakall, R. and Sydes, M. (2000). Achieving practical outcomes from genetic studies of rare Australian plants. *Australian Journal of Botany* **48**, 375-382.

Acknowledgements

This project was funded by the Department of Employment, Education, Training and Youth Affairs, the Australian Research Council and the New South Wales National Parks and Wildlife Service through an Australian Postgraduate Award (industry) scholarship. Fieldwork was undertaken, with permission, on land managed by the New South Wales National Parks and Wildlife Service.

Many people have provided assistance throughout this research project and I would like to thank the following.

- Members of my supervisory panel, Dr Rod Peakall (Chair, primary supervisor), Dr Tony Auld (secondary supervisor) and Dr Siegy Krauz (advisor), for their guidance and support.
- Both Professor Andrew Cockburn (past Head of Division) and Associate Professor Mike Crisp (present Head of Division), for allowing me the use of facilities within the Division of Botany and Zoology at the Australian National University.
- Members of the *Zieria prostrata* recovery team, in particular, Andrew Steed, Steve Clemesha, Tony Auld and Rod Peakall, for being so interested and involved in this project.
- Steve Clemesha, John Wrigley and Alex Floyd, for providing information on the past management of *Zieria prostrata.*
- Mike Crisp, for his guidance with the taxonomic methodology and analysis.
- Marita Sydes and Mike Ramsey, for general assistance and helpful discussions.
- Wes Keys, Simon Gilmore and Jeanette Cashin, for assistance in the laboratory.
- The glasshouse staff at the Australian National University, for looking after my cultivated plants.
- Rod Peakall and Tony Auld, for their valuable comments on drafts of this thesis and Marita Sydes for proof reading the almost final draft.
- Friends in Canberra, in particular Marita, Patrick, Kaska, Nina and Paul, who made my time in Canberra a memorable one.
- Dad, Mum and Steve, for all their support and for providing me with a bed and home cooked meals while finally finishing this thesis.
- Last, but far from least, Mike for his continual support and encouragement.

Abstract

Given the large number of threatened plant species, the development and implementation of effective recovery programs is an urgent priority. Research can play an important role in the development of these recovery programs. However, given limited resources, and the large number of threatened species, it is neither possible, nor practical, to conduct research on all. Therefore, we need to identify when research is most likely to lead to management outcomes. The primary objective of this study was to investigate the population genetics, taxonomy and reproductive ecology of the endangered plant *Zieria prostrata* ms. (Rutaceae). A secondary objective was to evaluate the management outcomes arising from this research in order to gain insight into when research is most likely to assist in the management of threatened plant species.

Zieria prostrata is known from only four headlands along a three kilometre stretch of coastline in northern New South Wales, Australia. The species was also presumed to have occurred at a fifth headland, 24 kilometres south of its present range. Random Amplified Polymorphic DNA (RAPD) analysis was used to assess patterns of genetic variation within and among the extant populations. The analysis also included an individual reputedly rescued from the extinct population. Principal coordinate analysis (PCO) and an analysis of molecular variance (AMOVA) revealed a high level of population divergence. Unexpectedly, the individual apparently sampled from the now extinct population was found to be genetically similar to individuals from one of the extant populations. After further investigation, this finding led to the conclusion that prior existence of the species at the fifth site was doubtful and subsequently a proposed reintroduction program was abandoned.

Although *Z. prostrata* is widely accepted as a distinct species, a formal description has not yet been published. The discovery of another headland *Zieria* taxon during this study, *Z.* sp. aff. *smithii*, raised questions about the taxonomic status of *Z. prostrata* and its relationship to the *Z. smithii* species complex. A morphometric investigation was used as the primary tool for investigating the relationship between *Z. prostrata* and the *Z. smithii* species complex, while a genetic study utilising RAPD markers was used to assess the validity of the distinct evolutionary lineages implied by the morphometric analysis. Based upon the combined morphometric and genetic data set, *Z. prostrata* formed a separate group in phenetic space, distinct and discrete from *Z.* sp. aff. *smithii* and *Z. smithii,* and is thus worthy of specific status. In contrast, while the combined data set suggested *Z.* sp. aff. *smithii* is worthy of sub-specific status, the genetic data alone

revealed that each headland population is likely to have originated independently from inland populations of *Z. smithii.* Therefore, the morphological similarity among populations of *Z.* sp. aff. *smithii* does not reflect evolutionary relatedness, but rather is likely to be a consequence of parallelism and active or rapid speciation. *Zieria* sp. aff. *smithii* is therefore considered to be a headland ecotype of *Z. smithii.*

The reproductive ecology of *Z. prostrata* was investigated to determine if there were any factors limiting recruitment. Seed germination and seedling survival were found to be the critical life history stages limiting recruitment, and safe site availability was found to be the limiting factor. However, given that *Z. prostrata* is long-lived and that the adult populations appear stable, the current availability of safe sites is likely to be sufficient to maintain population stability, at least in the immediate future. Effective management of these populations will thus centre upon either: (i) maintenance of the current availability of safe sites if current population size is considered sufficient, or (ii) increasing safe site availability if an increase in population size is desired.

Evaluation of the outcomes arising from this research provided a number of lessons on the contribution of research to the management of threatened flora. In addition, it was apparent that while all research is likely to provide conservation implications, not all implications are guaranteed to lead to changes in management. If research is to assist in the effective management of threatened species to its full potential, it is vital that thought is given to the possible outcomes of research while prioritising research tasks both within and among species. The targeting of funds towards research projects that are most likely to lead to practical outcomes will surely not only improve the contribution of research, but also the effective conservation of threatened species.

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CHAPTER 1
Background, study species and thesis objectives

1.1 Background

The conservation of threatened plants has become an international imperative, with nearly 34 000 species, or roughly 12.5 % of the worlds vascular flora facing extinction (Walter and Gillett 1998). Within Australia we are equally concerned, with at least 64 plant species already lost to extinction and over 1 000 threatened plant species heading that way if no action is taken to reverse their decline (ANZECC 1999). The effective conservation of these threatened species is therefore an urgent priority.

The effective conservation of threatened flora, however, is not an easy task. Aside from the obvious difficulties associated with halting habitat destruction and minimising human induced pressures upon populations of threatened species, the task is further complicated by the large number of threatened species requiring attention and by the limited resources available to conservation managers. Given the large number of threatened species, considerable effort has gone into developing appropriate risk assessment protocols enabling prioritisation among species for recovery efforts (e.g. Akcakaya *et al.* 2000; Burgman *et al.* 1999; Keith 1998; Keith *et al.* 2000). For those species subsequently targeted for recovery efforts, it is vital that the recovery programs themselves are cost efficient and effective.

Research is widely recognised as playing an important, if not crucial, role in the development of such effective recovery programs. Population genetics, taxonomy and reproductive ecology are three broad areas of research that can assist in the recovery of threatened plant species.

Genetic research is typically applied to the recovery of threatened plant species for two main reasons. Firstly, genetic research is frequently undertaken in an attempt to assist in the design of both *ex-situ* and *in-situ* management programs to ensure they maintain genetic diversity. Populations of threatened species are typically small and isolated and are therefore potentially susceptible to a loss of genetic diversity through both genetic drift and inbreeding (Barrett and Kohn 1991; Ellstrand and Elam 1993). Given that low levels of genetic diversity are thought to reduce the ability of populations to adapt to changing environments and to survive (Frankel and Soule 1981; Schaal *et al.* 1991), the maintenance of genetic diversity is one of the key goals of recovery programs. Secondly, genetic research is also a valuable tool when applied to the design of

translocation programs (Australian Network for Plant Conservation 1997b; Fenster and Dudash 1994; Guerrant-Jr 1992; Havens 1998; Mistretta 1994), or any other management activity that may lead to the mixing of genetically distinct individuals. The consequences of mixing distinct gene pools are largely unknown, however, one concern is outbreeding depression, a reduction in fitness associated with the mating of genetically distinct individuals (Templeton 1986).

Taxonomic research can also play an important role in the management of threatened plant species, particularly when the taxonomic status of a taxon is uncertain, as decisions regarding the delineation of species have important implications for conservation and management (Hibbett and Donoghue 1996; Hopper 1993; Shapcott 1998; Soltis and Gitzendanner 1999). Inappropriate taxonomy may lead to a failure to recognise and thus conserve threatened species. Such a failure to recognise species not only impinges upon the conservation of the species concerned, but also upon the conservation status of the associated plant and animal community, and thus reserve acquisition (Hopper 1993; Rojas 1992; Shapcott 1998). Alternatively, inappropriate taxonomy may lead to greater priority being placed upon a taxon than deserved. Given the reality of meagre resources available for conserving a multitude of threatened species, it is important to ensure funds are directed to the most appropriate taxa.

Research into the demography or reproductive ecology of threatened plant species is widely regarded as being an essential component of effective management (Griggs and Jain 1983; Pavlik and Manning 1993; Schemske *et al.* 1994; Synge 1981). An understanding of the reproductive ecology and critical life history stages of a threatened plant population can provide insight into possible limits facing a population and population stability. Such understanding can reveal whether management measures to increase population size and stability are necessary. The identification of limits to reproduction can also help identify management options which may be used to overcome these limits and to thus potentially stabilise or increase population size (e.g. Hegazy 1990; Lesica 1992; Morgan 1995; Pavlik *et al.* 1993; Pavlik and Manning 1993).

Despite the value of research, given the large and increasing number of threatened species and the limited resources available for research, it is neither possible, nor practical, to conduct research on all threatened species. Nor is it practical, or necessary, for example, to undertake population genetic, ecological and taxonomic research for each threatened species. Managers need to be able to prioritise among research tasks both within and among species. Therefore, in order to achieve effective and cost efficient recovery of threatened plant species, we need to identify when research, and

what form of research, is likely to produce practical outcomes for conservation management (Hogbin and Peakall 1999; Hogbin *et al.* 2000; Peakall and Sydes 1996). This may best be achieved by assessing and critically evaluating the outcomes of research for those species for which detailed research has been possible. The endangered plant *Zieria prostrata* provides one such case study.

1.2 The study species: *Zieria prostrata* ms. (Rutaceae)

The prostrate shrub, *Zieria prostrata* ms. (Rutaceae) possesses a highly restricted distribution and is known from only four coastal headlands along a mere three kilometre stretch of coastline near Coffs Harbour in northern New South Wales, Australia (Figure1.1). Although it is difficult to estimate exact population sizes owing to the prostrate and intertwining habit of the species, these four populations are likely support more than 1 000 individuals, with sites 1, 2, 3 and 4 supporting approximately 800, 9, 200 and 100 individuals respectively (NPWS 1998). The species was also presumed to have occurred at a disjunct fifth headland (site 5), 24 kilometres south of its present distribution (Griffith 1992; Figure 1.1).

Zieria prostrata typically occurs among dwarf headland vegetation with a southern aspect. The vegetation is characterised mainly by *Themeda australis* grassland and dwarf heathland species including: *Helichrysum bracteatum, Hibbertia vestita, Oxylobium scandens* var. *obovatum*, *Pultenaea* sp. aff. *villosa, Pimelia linifolia*, *Senecio lautus* subsp. *maritimus* and *Hydrocotyle peduncularis*. On more sheltered aspects, *Zieria prostrata* also associates with wind-pruned open to sparse shrubland characterised by the species *Banksia integrifolia* var. *integrifolia* and *Acacia sophorae.*

Like many *Zieria* species, although widely regarded as a distinct species, a description of this taxon has not yet been formally published. The taxon is referred to as *Zieria* species Q in the Flora of New South Wales (Armstrong 1991) and is listed as an endangered species under the name *Zieria prostrata* ms. at both a State (*Threatened Species Conservation Act 1995*) and National (*Environmental Protection and Biodiversity Conservation Act 1999*) level.

A history of the management of *Z. prostrata* is outlined in Hogbin and Peakall (2000). In brief, the first recovery plan for *Z. prostrata* was released in 1992 and was prepared under the then Australian Nature Conservation Agency (ANCA) Endangered Species Program (Griffith 1992). Very little was known of the biology of the species at the time of writing the initial recovery plan. The species was known to produce abundant flowers and heavy seed crops (Griffith 1992), but was thought to be male sterile (Armstrong ms). Populations were thought to be dominated by older individuals and seedlings were

considered rare (Griffith 1992). The recovery plan recognised extensive habitat degradation caused by uncontrolled vehicle access, pedestrian activity and weed invasion as the main threats to the survival of the species. The proposed management actions centred upon habitat restoration and reservation, the establishment of an *ex-situ* collection and translocation. The proposed research tasks included both ecological and genetic research.

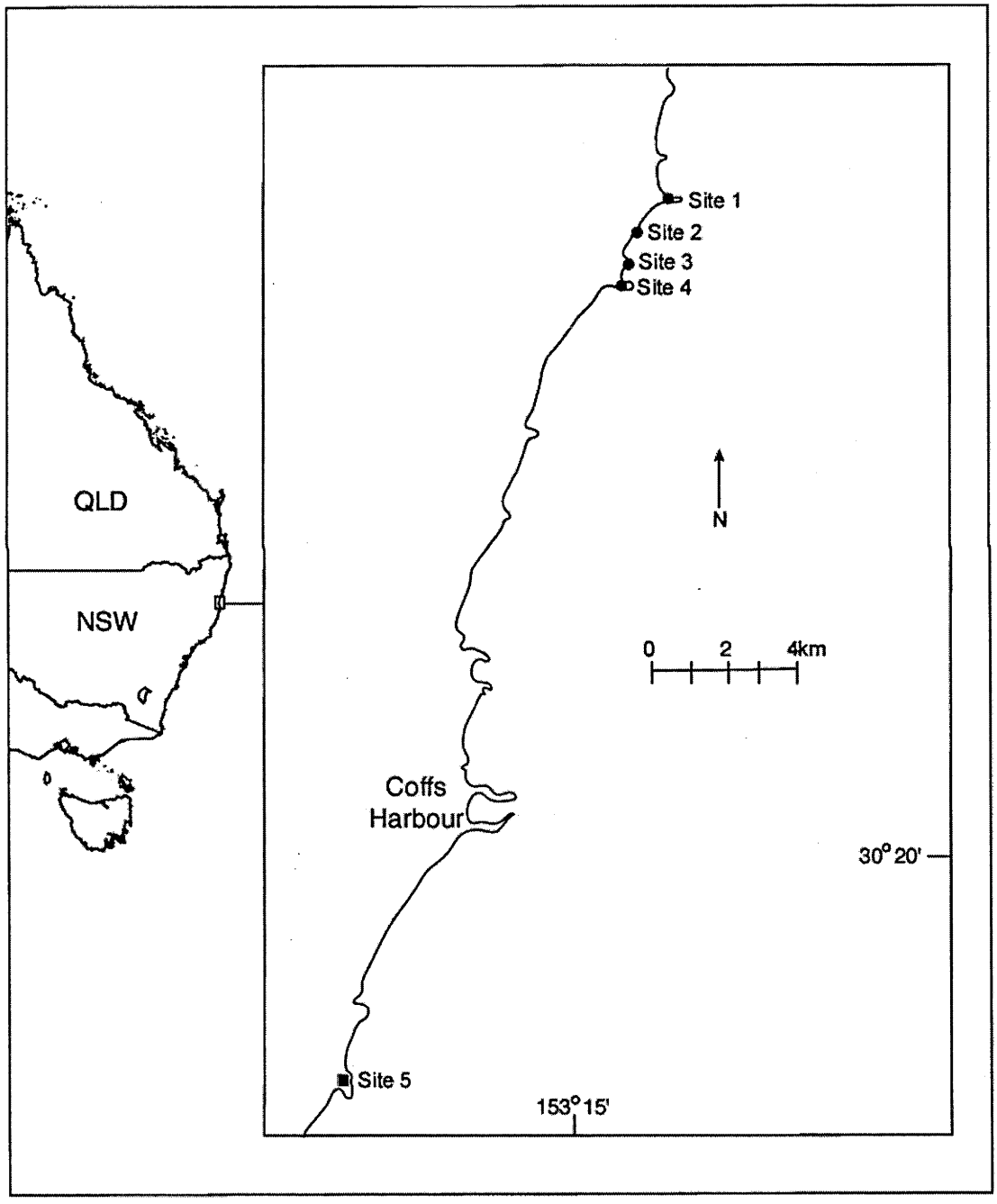

Figure 1.1 Distribution of *Zieria prostrata* depicting the location of the extant populations (●) and the location of the apparently extinct population (■).

An *ex-situ* collection of *Z. prostrata* was established in 1992 'as a safeguard against possible future losses from the wild' (Griffith 1992). Unfortunately, much of the process was poorly documented (Hogbin and Peakall 2000). It is known that cuttings were collected from two or three of the extant populations (Sites 3 and 4 and possibly site 1) and also from an individual reputedly rescued from the apparently extinct site 5 population. It is not known how many plants were sampled, nor from which plants the cuttings were taken. These cuttings were then propagated and maintained by the Coffs Harbour City Council. No record of the origin and success of individual cuttings was maintained. This *ex-situ* collection was maintained until 1996, when it was discarded due to its poor condition and failure to adequately fulfil its objective of being genetically representative, given that only two or three of the four populations were represented and no information on the number of genetic individuals was available.

Prior to disposal of the *ex-situ* collection, in 1993 it was used as a source of stock for a translocation program. Plants were reintroduced to site 5 and population enhancement occurred within two of the extant populations (sites 3 and 4) (S. Clemesha pers. comm.). Translocated plants were not individually tagged and no map of the translocation was made, nor are the numbers of plants involved known. The success of the translocated plants was not monitored. Subsequent evaluation of the translocation program was hampered by this poor documentation. By interviewing individuals involved in the initial program at the site itself, I estimate that sites 3 and 4 were enhanced with 50 and 150 individuals respectively. At most, 19 (12.7%) of the translocated plants were still surviving in 1998 at site 4 and only 5 (10%) at site 3. It is not known how many plants were reintroduced to site 5, with only one surviving plant relocated.

In 1995 all four known extant populations of *Zieria prostrata* were included within the Moonee Beach Nature Reserve. This achievement was largely a result of submissions from the local Ulitarra Conservation Society (S. Clemesha pers. comm.), political interest, and strong local support for the reservation of the headlands. This strong support was not solely due to the presence of *Z. prostrata*, and can also be attributed to a proposal for a sewerage outfall off site 4. The responsibility for management of the headlands was thus passed from the Coffs Harbour City Council to the NSW National Parks and Wildlife Service (NSW NPWS). The current study commenced in 1996 soon after the NSW NPWS took over the responsibility of managing the headlands.

1.3 Thesis objectives

The overall objectives of this study were twofold. The first objective was to investigate the population genetics, taxonomy and reproductive ecology of *Zieria prostrata*. The second objective was to evaluate the practical outcomes arising from this research in order to gain greater insight into when research is most likely to contribute to the effective conservation of threatened plant species.

More specifically, the objectives of this research project were to:

1. Investigate the population genetic structure of *Zieria prostrata* in order to assist in the design of a translocation program involving reintroduction to site 5 and enhancement of the extant populations (Chapter 2).

2. Clarify the taxonomic status of *Z. prostrata* by analysing both morphological and genetic variation among populations of *Z. prostrata* and the widespread *Z. smithii* species complex (Chapter 3).

3. Investigate the reproductive ecology of *Z. prostrata* in order to determine if there are any factors limiting recruitment, and to identify management options capable of increasing recruitment if necessary (Chapter 4).

4. Evaluate the practical outcomes arising from research into the population genetics, taxonomy and reproductive ecology of *Z. prostrata*, and more broadly to assess the implications of findings for conservation management in general (Chapter 5).

CHAPTER 2
Population genetics of the extant populations of *Zieria prostrata* and their relationship to a presumed extinct population

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2.1 Introduction

2.1.1 Background

One goal of conservation is the preservation of genetic diversity, as a loss of genetic variation is thought to reduce the ability of populations to adapt to changing environments and to survive (Barrett and Kohn 1991; Frankel and Soule 1981; Schaal *et al.* 1991). This concern for the loss of genetic diversity has led to an increase in the application of genetic knowledge to the design of conservation strategies for rare plants (Falk and Holsinger 1991; Fenster and Dudash 1994). For example, genetic knowledge has been applied to; the design of sampling strategies for *ex-situ* collections and translocation activities (e.g. Ceska *et al.* 1997; Wolf and Sinclair 1997), reserve design (e.g. Coates and Sokolowski 1992; Sampson *et al.* 1988), the assessment of a reintroduction program (Robichaux *et al.* 1997), and to the assessment of the conservation value or status of *ex-situ* collections (e.g. Calero *et al.* 1999; Ibanez *et al.* 1999; Knapp and Connors 1999) and natural populations (e.g. Hogbin *et al.* 1998; Prober *et al.* 1990).

The current genetic study of *Zieria prostrata* was proposed by the NSW National Parks and Wildlife Service (NSW NPWS) to assist in the design of a translocation program. The primary objective of the proposed translocation program was to reintroduce *Z. prostrata* to the apparently extinct site 5. A secondary objective was to enhance the extant populations (Griffith 1992; S. Clemesha pers. comm.). As outlined in Section 1.2, a preliminary, largely unsuccessful translocation program was implemented in 1993. It was envisaged this genetic research would assist in the design of a second, larger, and hopefully more successful translocation program.

2.1.2 Genetic considerations for translocation

The importance of applying genetic information to the successful implementation of translocation programs has been widely accepted (Australian Network for Plant Conservation 1997b; Fenster and Dudash 1994; Guerrant-Jr 1992; Havens 1998; Mistretta 1994). Given the distinct concerns relevant to the reintroduction of site 5 and

the enhancement of the extant populations, the following discussion addresses these two aspects of the translocation program independently.

2.1.2.1 Genetic considerations for the reintroduction of site 5

The main objective of the reintroduction program proposed by the NSW NPWS was to create a viable, self-sustaining population of *Zieria prostrata* at site 5. Aside from the obvious ecological and demographic considerations, one criterion considered vital for a self-sustaining population is the possession of sufficient genetic variation to allow adaptation to disease pressures and changing environmental conditions and to thus allow evolutionary development (Barrett and Kohn 1991; Frankel and Soule 1981; Franklin 1980; Huenneke 1991).

The presence of adequate genetic diversity in the reintroduced population may also decrease the chances of inbreeding and thus potential inbreeding depression, a reduction in fitness associated with mating among close relatives and the resultant increase in homozygosity (Charlesworth and Charlesworth 1987, 1999). There are two alternate, although not necessarily independent, models for the genetic basis of inbreeding depression (reviewed by Charlesworth and Charlesworth 1987, 1999). The first, and most favoured, is that inbreeding depression is due to the expression of recessive or partly recessive deleterious alleles in the homozygous state. The second model assumes heterozygote superiority. Inbreeding depression may manifest itself in higher proportions of embryo abortion, lower growth rates, higher mortality of both seeds and individual plants and lower probability of flowering (Barrett and Kohn 1991; Charlesworth and Charlesworth 1987). Obviously, such negative impacts on fitness can have important implications for both the short- and long-term viability of reintroduced populations.

The choice of stock used to create the reintroduced population is therefore critical to creating a population possessing sufficient genetic variability to maintain its fitness and long-term evolutionary potential. For reintroductions, it is usually preferable to use propagules from the site to be reintroduced if they exist in sufficient quantity and genetic diversity (Guerrant-Jr 1992; Reinartz 1995). Propagules from site 5 apparently existed, but were lacking genetic diversity, with the representation of only a single genotype. Prior to extinction of the site 5 population, cuttings were apparently collected from a single plant and propagated at the Australian National Botanic Gardens in Canberra. Subsequently, a small number of these plants were transferred to the Coffs Harbour Botanic Gardens and used as source plants for the *ex-situ* collection (J. Wrigley and S. Clemesha pers. comm.).

Given the lack of a genetically diverse local source, the source plants to be used in the reintroduction of site 5 required consideration. The single local genotype could be used as the sole source, or alternatively, propagules from a single non-local population, or from multiple non-local populations, could be used. There are both advantages and disadvantages associated with using either local or non-local source stock, or using single or multiple sources, as stock for a reintroduction (Barrett and Kohn 1991; Huenneke 1991).

2.1.2.1.1 Use of a single local genotype as the source for reintroduction

Arguments in favour of using the single local genotype as source for the reintroduction centre upon maintaining the integrity of stock that is specifically adapted to local environmental conditions. Plant reciprocal transplant experiments have consistently demonstrated that propagules transplanted into the microhabitat from which they were collected often have higher survival, growth and fecundity, than those moved to new localities (Bradshaw 1984; Chapin and Chapin 1981; McGraw and Antonovics 1983; Schmidt and Levin 1985; Waser and Price 1985).

Reintroduction of *Zieria prostrata* to site 5 however, was complicated by the availability of only a single local genotype. Even if that genotype is locally adapted to the current environmental conditions, what is the likely long-term survival of a population devoid of genetic diversity? Low levels of genetic variation do not necessarily mean that reintroduction attempts could not succeed in the short-term (Maunder 1992). For example, Schwaegerle and Schaal (1979) describe a thriving population of over 100 000 pitcher plants (*Sarracenia purpurea*) originating from a single translocated individual. As mentioned, however, an absence of genetic diversity in the founding gene pool may jeopardise the long-term persistence and evolutionary potential of the reintroduced population. Insight into the potential problems facing a reintroduced population created using the single local genotype can be gained by considering several historic disastrous crop failures (reviewed in Adams *et al.* 1998). For example, a frequently cited case is that of Ireland's potato (*Solanum tuberosum*) famine of the 1800's. Irelands entire potato crop was wiped out owing to a lack of resistance to *Phytophthora infestans,* the late blight fungus. The lack of resistance can be traced to the lack of genetic diversity in Irish potatoes (Hawkes 1979 as cited in Adams *et al.* 1998). It is therefore likely, that even if a reintroduced *Zieria prostrata* population large enough to survive demographic stochasticity could be created using the single local genotype, its long-term persistence in the face of changing environmental conditions and disease pressures could not be assured.

The presence of adequate genetic variation might also be especially critical for the founding plants used in the reintroduction of site 5 as the habitat of the headland has been greatly modified in recent times. The headland is now heavily infested with the exotic Kikuyu grass (*Pennisetum clandestinum*) and is highly disturbed due to pedestrian activity and the presence of a lookout and car park. It is therefore highly likely that the original habitat no longer exists. If local adaptation was present historically, the current environment has been so greatly modified that the local genotype may be no better adapted than plants from other headlands. Major habitat modification of potential reintroduction sites is presumably a frequently encountered problem. For example, Knapp and Connors (1999) found that the biotic environment of a potential reintroduction site for the endangered plant *Trifolium amoenum* had been greatly altered by exotic plant species and cattle grazing. When reintroductions are to occur in such novel or highly modified environments, greater variability in the reintroduced population may increase the chances that at least some individuals will be successful (Huenneke 1991).

The mating system of *Zieria prostrata* is also relevant to the success of a reintroduced population created using the single local genotype. If *Z. prostrata* is self-incompatible, the reintroduction of plants representing a single genotype could not produce a self-sustaining population because seed set would be absent. The absence of seed set, or reduced seed set, in small, genetically depauperate populations of plants with self-incompatibility systems has been well documented (Demauro 1994; Godt and Hamrick 1999; Les *et al.* 1991; Negron-Ortiz 1998; Thien *et al.* 1983). If, on the other hand, *Z. prostrata* is self-compatible, seed set could occur and concerns about the potential for inbreeding depression would lessened. Inbreeding depression is expected to be greater in outcrossing than in selfing populations, because there is a greater opportunity to purge deleterious alleles with high levels of selfing (Barrett and Charlesworth 1991; Charlesworth and Charlesworth 1987). Therefore, if the now extinct site 5 *Zieria prostrata* population was historically primarily selfing, the majority of deleterious alleles may have been purged, and maintaining high levels of genetic diversity in order to avoid inbreeding depression may not be as vital.

2.1.2.1.2 Use of one or more non-local populations as sources for the reintroduction

Given the potential limitations associated with using the single local genotype as the sole source for the reintroduction, the genetic base of the reintroduced population could be expanded by utilising propagules from one or more non-local populations. Arguments for using a single source population usually centre upon avoiding the mixing of genetically distinct individuals. The consequences of mixing distinct gene pools are largely unknown, however a frequently voiced concern is outbreeding depression, a

reduction in fitness associated with the mating of genetically distinct individuals (Templeton 1986).

Outbreeding depression may be caused by the disruption of co-adapted, or locally adapted, gene complexes (Templeton 1986). Although outbreeding depression has been reported in several plant species (e.g. Fischer and Matthies 1997; Parker 1992; Waser and Price 1989, 1994), how widespread this phenomenon is and the degree to which it should be considered when making decisions about whether to mix populations for conservation purposes is still subject to debate (Fenster and Dudash 1994). Caution against mixing genetically distinct populations is a common management recommendation arising from genetic research on rare plants (e.g. Godt and Hamrick 1996; Kress *et al.* 1994; Martin *et al.* 1997; Odasz and Savolainen 1996; Palacios and Gonzalez-Candelas 1997b; Travis *et al.* 1996; Wolf and Sinclair 1997).

Given the perceived concerns associated with mixing populations, the first choice for the reintroduction of site 5 may be the use of a single source population. Careful matching of the source population to the reintroduction site is appropriate where feasible (Charlesworth and Charlesworth 1987). Unfortunately, matching a source population to the reintroduction site based upon habitat similarities is unlikely given the highly modified environment of site 5. It may therefore be best to select a single source population which is most similar genetically to the rescued site 5 genotype.

The genetic diversity of the potential source populations requires investigation prior to deciding to use only a single source. Perhaps all non-local populations are genetically depauperate and thus a single source may not be genetically diverse enough. Maybe the extant populations are themselves only fragmented remnants of what they were historically. When using non-local source, genetic diversity becomes even more important. Translocated populations may not be well adapted to the area in which they are introduced and high genetic diversity can increase the chances of at least some genotypes surviving.

Given the potential limitations associated with using a single source, assessing the potential of a mixed source reintroduced population suffering outbreeding depression appears to be central to determining whether a single or mixed source stock is preferable (Guerrant-Jr 1992). An understanding of gene flow among the extant populations can provide insight into the likelihood of outbreeding depression occurring upon population mixing. If the four extant populations of *Zieria prostrata* are acting as a single large interacting population connected by gene flow, then the development of locally and co-adapted gene complexes will be unlikely as gene flow disrupts local

adaptation. The mixing of such populations is theoretically unlikely to result in outbreeding depression. On the other hand, if the extant populations are acting as a series of genetically isolated populations, then the evolution of locally and co-adapted gene complexes is likely, and thus outbreeding depression may be possible upon mixing.

2.1.2.2 Genetic considerations for the enhancement of the extant populations

The main objective of the enhancement of the extant populations of *Zieria prostrata* was to increase population stability through an increase in population size. There are two alternate approaches which could be taken to enhance population size. Firstly, adding local genotypes could increase census population size, or secondly, adding non-local genotypes could increase effective population size and genetic diversity. Arguments for enhancement using local genotypes centre upon avoiding the mixing of genetically distinct individuals and thus potential outbreeding depression (see Section 2.1.2.1.2). Whereas arguments for enhancement using non-local genotypes centre upon increasing population genetic diversity and thus evolutionary potential (see Section 2.1.2.1). The most appropriate approach will depend upon the distribution of genetic diversity within and among populations. As outlined in the previous section, if little differentiation occurs among the four extant populations and they act more or less as one continuous population connected via gene flow, then the expression of outbreeding depression upon enhancement using non-local genotypes is unlikely. Alternatively, if a high level of population differentiation is apparent, implying a low level of gene flow among populations, then population enhancement using non-local genotypes may theoretically lead to outbreeding depression.

2.1.3 Chapter objectives

The primary objective of this study was to investigate the population genetics of *Zieria prostrata* in order to assist in the design of a translocation program involving reintroduction to site 5 and enhancement of the extant populations.

More specifically, this genetic study addressed two main questions.

1. From a genetic perspective, does this highly endemic species consist of a single interacting population or a series of genetically isolated populations? It was envisaged such insight would reveal whether the mixing of populations should be avoided during reintroduction and population enhancement.

2. How does the genotype of the individual reputedly rescued from the now extinct population (site 5) compare with individuals from the extant populations? Such knowledge can highlight potential source populations for the reintroduction of site 5.

2.2 Methods

The genetic marker Random Amplified Polymorphic DNA (RAPD) was chosen to investigate the population genetic structure of *Zieria prostrata*. The RAPD technique (Williams *et al.* 1990) has been used extensively to investigate the population genetics of threatened plants (e.g. Ayres and Ryan 1997; Brunell and Whitkus 1997; Calero *et al.* 1999; Dowe *et al.* 1997; Hogbin *et al.* 1998; Maguire and Sedgley 1997; Martin *et al.* 1999; Palacios and Gonzalez-Candelas 1997a, 1997b; Rossetto *et al.* 1995; Sydes and Peakall 1998). The suitability of the RAPD technique for investigating the population genetics of threatened plants can be attributed to two main reasons. Firstly, the technique is highly sensitive to detecting genetic variation as a result of the possibility of scoring individuals at an essentially unlimited number of loci (Lynch and Milligan 1994; Welsh and McClelland 1990; Williams *et al.* 1990). This ability to detect high levels of polymorphism is particularly advantageous when working with rare plants, as some rare plants have been found to exhibit low or no detectable allozyme variation (e.g. Kress *et al.* 1994; Lesica *et al.* 1988; Waller *et al.* 1987; Wong and Sun 1999). Secondly, the RAPD technique is simpler, less costly, and less labour intensive than other DNA marker methodologies (Caetano-Anolles *et al.* 1991a, 1991b; Hadrys *et al.* 1992). One reason for this ease of use is that the RAPD technique requires no prior sequence information (Hadrys *et al.* 1992; Welsh and McClelland 1990; Williams *et al.* 1990). This is due to two major modifications; (i) single short primers (usually 10 bases) of arbitrary sequence are used instead of two specific primers (commonly 18-25 bases), and (ii) the annealing temperature is lowered. These two modifications lower the specificity of the reaction so that a number of anonymous but reproducible fragments can be amplified (Williams *et al.* 1990).

Despite its advantages, the RAPD technique possesses three potential limitations which require consideration. Firstly, one major concern with the use of RAPD markers is the run to run reproducibility of amplification profiles owing to the low stringency annealing conditions used in the PCR reaction and the resulting PCR artefacts (Hadrys *et al.* 1992; Peakall 1997). Problems with reproducibility, however, can be minimised if PCR reaction conditions are optimised and standardised, if the appropriate positive and negative controls are included, and the appropriate primers are selected (Hadrys *et al.* 1992; Morell *et al.* 1995; Rafalski and Tingey 1993). Secondly, some authors have questioned whether co-migrating fragments are necessarily homologous (Hurme and Savolainen 1999; Quiros *et al.* 1995; Williams *et al.* 1993). However, problems with non-homologous similar sized fragments are usually limited when comparisons are made within and between closely related taxa (Rieseberg 1996). The third potential limitation of the technique is the dominant nature of RAPD markers (Williams *et al.*

1990). This results in homozygotes being indistinguishable from heterozygotes (i.e. both possess band present phenotype). The dominant nature of RAPD markers means they provide less genetic information than co-dominant markers such as allozymes or microsatellites, although this limitation is partly offset by the larger number of bands that can be generated and does not lessen the suitability of the technique for population studies (Parker *et al.* 1998; Peakall 1997).

2.2.1 Sampling strategy

Fresh leaf material from 81 individuals from across all four known extant populations of *Zieria prostrata* was sampled for genetic analysis (Figure 1.1, Chapter 1). The structure of populations and differences in population size prevented comparable sampling strategies. The populations at sites 1 and 3 each form one continuous patch and 24 individuals were sampled at regular intervals from a single transect spanning each of these sites. In contrast, site 4 contains four distinct sub-populations, three of which were represented, along with an isolated individual, in the total sample of 24 individuals. The fourth sub-population was not discovered until after genetic analysis. All nine individuals were sampled from the smallest population at site 2. Plants known to have been planted into the populations during the 1993 population enhancement program (Section 1.2) were avoided during sampling. Additionally, the plant reputedly rescued prior to extinction of its population (site 5), and now growing in the Coffs Harbour Botanic Gardens, was sampled, as well as an individual cultivated from this source and reintroduced to site 5.

2.2.2 DNA extraction

Template DNA was isolated using a modified version of the technique applied by Huff *et al.* (1993). Fresh leaf tissue (1 g) was ground in liquid nitrogen. 0.6 ml of the ground tissue was added to 500 µl of extraction buffer (100 mM Tris, 50 mM EDTA, pH 7.5, 100 mM NaCl, 1% SDS, 10 mM mercaptoethanol and 0.1% PVP), briefly vortexed and incubated at 65°C for 15 minutes. 250 µl of 5 M potassium acetate was added, tubes were vortexed thoroughly, incubated on ice for 5 minutes, and then centrifuged at 13 000 rpm for 20 minutes. The supernatant was collected and 1/5 volume of 10 M ammonium acetate and 1 volume of -20°C isopropanol was added to the supernatant to precipitate DNA. Tubes were gently mixed and stored at -20°C for 20 minutes. The DNA was pelleted by centrifuging at 13 000 rpm for 10 minutes, washed in 70% ethanol, and re-suspended in 50 µl of low TE buffer (10 mM Tris-HCl, pH 8.0; 1 mM EDTA). DNA concentration was then standardised to 5 ng/µl by checking concentrations on 1% agarose gels and staining with ethidium bromide. Purity of DNA was assessed by restriction digestion with AluI. All samples digested successfully.

2.2.3 RAPD-PCR procedure

The RAPD polymerase chain reaction (PCR) (Williams *et al.* 1990) conditions followed those of Sydes and Peakall (1998) with minor modifications. Briefly, RAPD amplification reactions were 10 µl in volume and consisted of 1.0 µl of Perkin Elmer 10 X PCR reaction buffer (100 mM Tris-HCl, pH 8.3, 500 mM KCl, and 25 mM $MgCl_2$), dNTP's at 200 µM, Operon RAPD primer at 100 µM, 0.5 unit of AmplitaqDNA polymerase (Perkin Elmer) and 10 ng of template DNA. A Corbett Research Fast Thermal Sequencer (FTS-960) was used to amplify RAPD-PCR products according to the following program; 5 minutes at 94°C, 2 minutes at 35°C and 1.5 minutes at 72°C, 4 cycles of 10 seconds at 94°C, 2 minutes at 35°C and 1 minute at 72°C and 35 cycles of 10 seconds at 94°C, 25 seconds at 40°C and 1.5 minutes at 72°C followed by a final extension cycle for 5 minutes at 72°C.

In an initial survey, one hundred decamer primers (Operon Technologies Inc, California. Kits: OPA, OPB, OPK, OPAA and OPAB) were evaluated for suitability. One *Z. prostrata* individual was screened with all 100 primers. Of these 100 primers, 24 showed potentially useful profiles. To screen for polymorphisms, these 24 primers were screened further with one individual from each of the four populations. Of these 24, eight primers (OPA 1, OPA 5, OPA 12, OPB 7, OPB 8, OPB 10, OPK 4, and OPK 15) were found to reveal polymorphic bands that were reproducible across multiple runs. These eight primers were used to generate RAPD profiles for all 83 *Z. prostrata* samples.

To ensure reproducibility of the RAPD profiles, reaction conditions and template DNA concentration were closely controlled. For each set of PCR reactions, a negative control PCR reaction, which contained all ingredients except DNA, was included to check for contamination of the stock chemicals. In addition, all DNA samples were run with each primer at least twice to check for reproducibility of profiles among runs.

Amplification products were resolved electrophoretically on 1% agarose gels, stained with ethidium bromide and photographed on an ultraviolet transilluminator. The RAPD profile for each individual was scored directly from photographs of the gels by assigning a value of 1 for band presence and 0 for band absence. Monomorphic markers were excluded from subsequent analyses.

2.2.4 Data analysis

2.2.4.1 Investigating variation in marker frequency among populations

Bands that differed significantly in frequency among populations were identified using chi-square (χ^2) heterogeneity tests (Zar 1984). Given that each marker was tested

independently, resulting in 20 separate χ^2 tests, there is a strong chance at $P < 0.05$ that at least one type 1 error (rejecting H_0 of homegeneity of markers when it should be accepted) will occur by chance alone. Therefore it should be noted that marker heterogeneity across populations may be slightly inflated.

2.2.4.2 Pairwise genetic distance matrix

A pairwise Euclidean distance matrix (Excoffier *et al.* 1992; Huff *et al.* 1993) was generated from the presence-absence data (RAPDistance program; Armstrong *et al.* 1994). The Euclidean distance measure equates to a tally of band differences between individuals. There are numerous distance measures available (see Maguire and Sedgley (1997) for a comparison of distance measures), however, the Euclidean measure by Excoffier *et al.* (1992) is most appropriate for the subsequent analysis of molecular variance (AMOVA) analyses (Huff *et al.* 1993).

2.2.4.3 Investigating the cause of duplicated multilocus genotypes

Observation of the distance matrix revealed the occurrence of duplicated multilocus genotypes, primarily within site 4. Duplicated multilocus genotypes are expected within a given data set when the genetic markers fail to provide sufficient resolution, or when plants are unexpectedly large, clonal, or exhibit high levels of selfing. It is clearly important to differentiate among these alternatives, since failure to recognise large plant size or clonality can result in inappropriate statistical analysis of population structure. Estimation of the resolving power within the data set was performed for site 4 only, following the methods provided by Sydes and Peakall (1998).

Briefly, the resolving power available within a given data set can be estimated by calculating the probability of detecting the number of copies of individual genotypes observed in the data set. This can be achieved by estimating the probability of identical genotypes arising through sexual reproduction. When using dominant markers the probability of the multilocus genotype is given by the product of the single locus genotype probabilities:

$P_{dgen} = \Pi p_i$ (Equation 1)

where p_i is the frequency of the band presence or absence observed at each locus in the multilocus genotype. Equation 1 calculates the probability of drawing a second copy of a particular genotype given that you have already drawn one copy of that particular genotype. The probability of observing a genotype the number of times that it is seen in the data (n) is given by the probability of drawing (n-1), given that one is in hand. That is;

P (drawing n copies of a genotype) = $(P_{dgen})^{n-1}$ (Equation 2)

where n is the number of times the genotype was observed. If $P<0.05$, then the null

hypothesis of random mating from a panmictic gene pool as the cause of the observed genetic patterns is rejected (i.e. insufficient resolving power does not explain the presence of duplicated genotypes).

2.2.4.4 Principal coordinate analysis

The pairwise Euclidean distance matrix (Section 2.2.4.2) formed the basis of examination of the patterns of genetic relationships through principal coordinate analysis (PCA) (SYN-TAX program; Podani 1995). The data was also analysed using non-metric two and three dimensional multidimensional scaling (NMDS). The NMDS revealed similar patterns to the PCA analysis and therefore the NMDS results are not presented here.

2.2.4.5 Analysis of molecular variance

The pairwise Euclidean distance matrix also formed the basis of examination of the distribution of genetic variability within and among populations using an analysis of molecular variance (AMOVA) (WINAMOVA program; provided by Laurent Excoffier, University of Bern, Switzerland). The AMOVA method, which was initially developed for Restriction Fragment length Polymorphism (RFLP) data (Excoffier *et al.* 1992), was first applied to the analysis of RAPD phenotypes by Huff *et al.* (1993). Since its initial application to RAPD phenotypes, the AMOVA method has been increasingly used to analyse RAPD data (e.g. Dawson *et al.* 1993; Dawson and Powell 1999; Fischer and Matthies 1998; Hogbin *et al.* 1998; Maguire and Sedgley 1997; Martin *et al.* 1999; Nesbitt *et al.* 1995; Rossetto *et al.* 1995; Vazquez *et al.* 1999). The suitability of the AMOVA technique for the analysis of dominant markers is based upon its lack of dependence upon assumptions about the distribution of the data (Morell *et al.* 1995) and its failure to inflate population differentiation, as can other methods of RAPD marker analysis (Isabel *et al.* 1999).

The AMOVA method produces analogs to Wright's *F*-statistics and is a powerful procedure for the analysis of genetic variation when there is hierarchical structure within the data set (Morell *et al.* 1995). AMOVA performs an analysis of variance within and among the different hierarchical levels of the data set and computes significance levels for variance component estimates by non-parametric permutational procedures. The number of permutations for significance testing was set at 1 000 for all analyses.

An AMOVA was used to estimate variance components attributable to differences within and between three of the extant populations: sites 1, 3 and 4. Site 2 was excluded from the AMOVA analysis due to the potential bias its small sample size may cause. A

second AMOVA analysis was also performed to investigate the distribution of genetic variability within and among the northern (site 1) and southern (sites 3 and 4) geographic regions (Fig 1.1, Chapter 1). These two analyses were then repeated after excluding repeated genotypes from the data set to ensure the presence of the repeated genotypes did not bias population differentiation estimates.

2.3 Results

2.3.1 The RAPD profile

In total, the eight RAPD primers produced 53 markers. Of these markers, 20 (37%) were polymorphic and informative (Table 2.1). Chi-square heterogeneity testing revealed that, of the 20 polymorphic markers, 13 (65%) were significantly heterogeneous across populations ($P < 0.05$), three of which were population specific for site 2 (Table 2.2).

2.3.2 The distance matrix and duplicated genotypes

Observation of the distance matrix revealed the occurrence of duplicated genotypes within, but not among, populations (Table 2.3). At sites 1 and 3, the RAPD profiles were highly discriminatory, with unique multilocus genotypes detected for all 24 individuals at site 1, and 22 of 24 individuals at site 3. At site 2, however, only two multilocus genotypes were detected among the nine individuals, and at site 4, only 12 unique multilocus genotypes were detected among the 24 individuals, with duplicated genotypes observed within all three of the sampled sub-populations (Figure 2.1). Estimating the resolving power within the data set revealed that the probability of identical genotypes arising via sexual reproduction was low ($0.02 - 2.46 \times 10^{-7}$) (Figure 2.1), indicating that inadequate genetic resolution does not account for the detection of duplicated genotypes. The presence of duplicated genotypes may therefore indicate either large plant size or non-random reproduction (e.g. extreme inbreeding or asexuality). Detailed examination in the field ruled out clonality and large plant size as explanations for the occurrence of duplicated genotypes. The most likely explanation for the repeated genotypes is therefore selfing within semi-isolated sub-populations. In all subsequent analyses all individuals were therefore treated as genetically distinct (i.e. repeated genotypes were not pooled).

2.3.3 Population differentiation

Principal coordinate analysis (PCA) revealed that the two multilocus genotypes that were detected within the smallest population at site 2 were clearly distinct from the remaining three populations (Figure 2.2). Noticeable population clustering corresponding to geographic relationships was apparent among the three remaining

populations. Site 1, the northern most population, formed a distinct cluster, largely separate from the two southern populations (sites 3 and 4) whose genotypes overlap in the PCA ordination space. Of particular interest is the position of the individual apparently sampled from site 5 prior to the population's extinction, which clusters amongst the extant populations, close to individuals from site 3.

An analysis of molecular variance (AMOVA) revealed considerable population differentiation, with 37% of the total variation being distributed among populations (Table 2.4). The remaining 63% represented variation among individuals within populations.

Given the overlap apparent among sites 3 and 4 in ordination space (Figure 2.2), an AMOVA was also performed to compare the northern population (site 1) with the combined southern populations (sites 3 and 4). Of the total variation, 35% was distributed among the northern and southern populations, and 65% occurred among individuals within geographic regions (Table 2.4). Thus the large population divergence exhibited within this species is attributable mainly to divergence among the northern and southern populations.

The pattern of extensive population divergence remained when duplicated multilocus genotypes were excluded from the analyses. Of the total variation, 35% was represented among the three populations and 33% was distributed among the northern and southern sites. This indicates that even if unintentional sampling of large plants had occurred, it did not affect the overall conclusions regarding population differentiation.

Table 2.1 Attributes of the eight oligonucleotide primers used for generating RAPD markers for 83 individuals of *Zieria prostrata* sampled from four populations.

Primer	5'-3' sequence	Number of markers scored	Number of polymorphic markers
OPA-1	CAGGCCCTTC	7	3
OPA-5	AGGGGTCTTG	7	3
OPA-12	TCGGCGATAG	4	2
OPB-7	GGTGACGCAG	7	3
OPB-8	GTCCACACGG	5	3
OPB-10	CTGCTGGGAC	11	2
OPK-4	CCGCCCAAAC	5	1
OPK-15	CTCCTGCCAA	7	3
TOTAL		53	20

Table 2.2 The frequency of all 20 polymorphic RAPD markers across the four populations of *Zieria prostrata.* [#] indicates a significant difference in marker frequency among populations (at $P < 0.05$) and [@] indicates fixed differences among populations.

Marker	Marker frequencies				
	Site 4 (n=24)	Site 3 (n=24)	Site 2 (n=9)	Site 1 (n=24)	Total
OPB10.1[#]	13	2	0	6	21
OPBB10.1	24	10	9	18	61
OPK15.1[#]	13	15	0	24	52
OPK15.2	12	20	9	24	65
OPK15.3[#@]	24	24	0	24	72
OPB7.1[#]	5	2	9	15	31
OPB7.2	0	3	0	4	7
OPB7.3[#]	0	3	9	7	19
OPK4.1[#]	0	0	9	1	10
OPA1.1[#]	0	1	0	7	8
OPA1.2	24	19	9	16	68
OPA1.3[#]	11	23	0	24	58
OPA5.1[#]	23	24	0	24	71
OPA5.2[#]	0	2	6	22	30
OPA5.3	15	15	9	13	52
OPB8.1[#]	16	24	0	0	40
OPB8.2[#@]	24	24	0	24	72
OPB8.3	24	17	9	24	74
OPA12.1[#@]	24	24	0	24	72
OPA12.2	24	24	9	22	79

Table 2.3 Euclidean distances between pairs of *Zieria prostrata* individuals within each of the four populations obtained using 20 RAPD markers. Duplicated genotypes are present within populations as revealed by a genetic distance of 0 (in bold).

Site 1	Site 2
0 4 0 6 4 0 7 7 3 0 3 5 3 4 0 5 3 3 6 4 0 4 4 4 7 5 1 0 4 6 4 7 3 3 2 0 5 3 7 8 6 4 5 5 0 3 5 5 6 2 2 3 3 4 0 2 4 6 7 3 3 4 4 3 1 0 4 6 1 0 9 7 7 6 6 3 5 4 0 3 5 7 8 6 4 3 3 2 4 3 3 0 3 5 7 6 4 4 5 5 2 2 1 3 2 0 3 3 5 8 4 2 3 3 2 2 1 5 2 2 0 3 5 7 1 0 6 4 3 3 4 4 3 3 2 4 2 0 4 6 8 7 5 5 6 6 3 3 2 2 3 1 3 3 0 4 4 6 7 5 3 4 4 1 3 2 4 1 1 1 3 2 0 3 3 5 6 4 2 3 5 4 2 1 5 4 2 2 4 3 3 0 5 5 5 8 4 4 5 3 4 4 3 5 4 4 2 2 3 3 4 0 2 4 4 5 1 5 6 4 5 3 2 6 5 3 3 5 4 4 3 3 0 7 5 5 8 4 4 5 3 4 4 5 5 6 6 4 4 5 5 6 2 5 0 6 6 6 9 5 3 4 4 5 3 4 6 5 5 3 3 4 4 5 3 6 3 0 3 7 7 8 4 6 5 3 6 4 3 5 4 4 4 4 5 5 4 4 3 6 5 0	0 0 0 0 0 0 1 1 1 0 1 1 1 0 0 1 1 1 0 0 0 1 1 1 0 0 0 0 1 1 1 0 0 0 0 0 1 1 1 0 0 0 0 0 0
Site 3	**Site 4**
0 2 0 5 5 0 2 2 5 0 7 7 6 7 0 5 3 4 5 8 0 3 3 4 5 6 2 0 4 4 3 4 5 3 1 0 3 3 2 3 4 6 4 3 0 6 6 5 8 7 5 3 4 5 0 4 6 5 4 7 5 3 2 5 6 0 4 6 5 4 7 5 3 2 5 6 0 0 1 3 4 3 8 4 2 3 4 5 3 3 0 4 6 5 4 7 7 5 4 5 8 2 2 3 0 5 5 4 5 6 4 2 1 4 5 1 1 4 3 0 4 4 3 4 5 3 1 0 3 4 2 2 3 4 1 0 3 5 2 3 6 6 4 3 2 5 3 3 2 3 4 3 0 .5 7 6 5 4 6 4 3 6 7 3 3 4 5 4 3 4 0 2 2 5 4 5 3 1 2 3 4 4 4 3 6 3 2 5 5 0 5 3 4 3 4 4 4 3 2 5 5 5 6 7 4 3 4 6 3 0 3 3 2 3 6 4 2 1 2 5 3 3 2 3 2 1 2 4 3 4 0 3 5 6 5 8 4 2 3 6 5 1 1 2 3 2 3 4 4 3 6 4 0 3 5 4 5 6 4 2 3 4 3 3 3 2 5 4 3 2 4 3 4 4 2 0 5 5 4 5 4 4 2 1 4 5 3 3 4 5 2 1 4 2 3 4 2 4 4 0	0 0 0 0 0 0 3 3 3 0 3 3 3 4 0 4 4 4 5 1 0 4 4 4 5 1 0 0 5 5 5 6 2 1 1 0 4 4 4 5 1 0 0 1 0 4 4 4 5 1 0 0 1 0 0 6 6 6 5 3 2 2 3 2 2 0 6 6 6 5 3 2 2 3 2 2 0 0 6 6 6 5 3 2 2 3 2 2 0 0 0 4 4 4 5 5 4 4 5 4 4 2 2 2 0 4 4 4 5 5 4 4 5 4 4 2 2 2 0 0 4 4 4 5 5 4 4 5 4 4 2 2 2 0 0 0 4 4 4 5 5 4 4 5 4 4 2 2 2 0 0 0 0 3 3 3 2 6 5 5 6 5 5 5 5 5 3 3 3 3 0 3 3 3 2 2 3 3 4 3 3 5 5 5 7 7 7 7 4 0 2 2 2 3 5 4 4 5 4 4 6 6 6 4 4 4 4 1 3 0 1 1 1 4 4 5 5 6 5 5 5 5 5 3 3 3 4 4 3 0 2 2 2 5 5 4 4 5 4 4 4 4 4 2 2 2 2 3 5 2 1 0 5 5 5 4 4 3 3 4 3 3 3 3 3 5 5 5 5 4 2 3 4 3 0 1 1 1 4 4 5 5 6 5 5 5 5 5 3 3 3 4 4 3 0 1 4 0

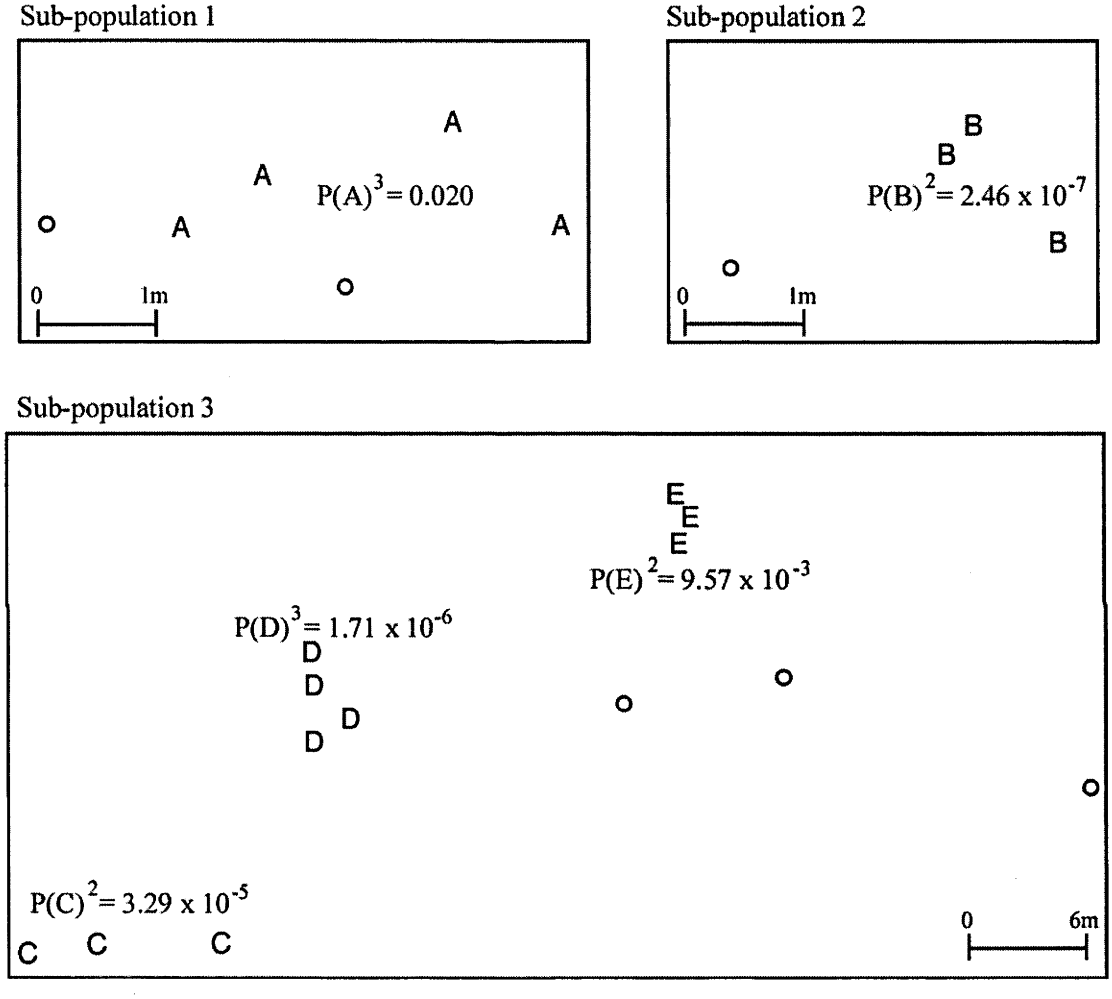

Figure 2.1 A map depicting 23 of the 24 *Zieria prostrata* individuals sampled from across three sub-populations at site 4. The circles represent distinct genotypes and each letter corresponds to a particular duplicated genotype. The probability of each of these genotypes arising independently, through sexual reproduction, the number of time observed (n) is provided $[(P_{dgen})^{n-1}]$.

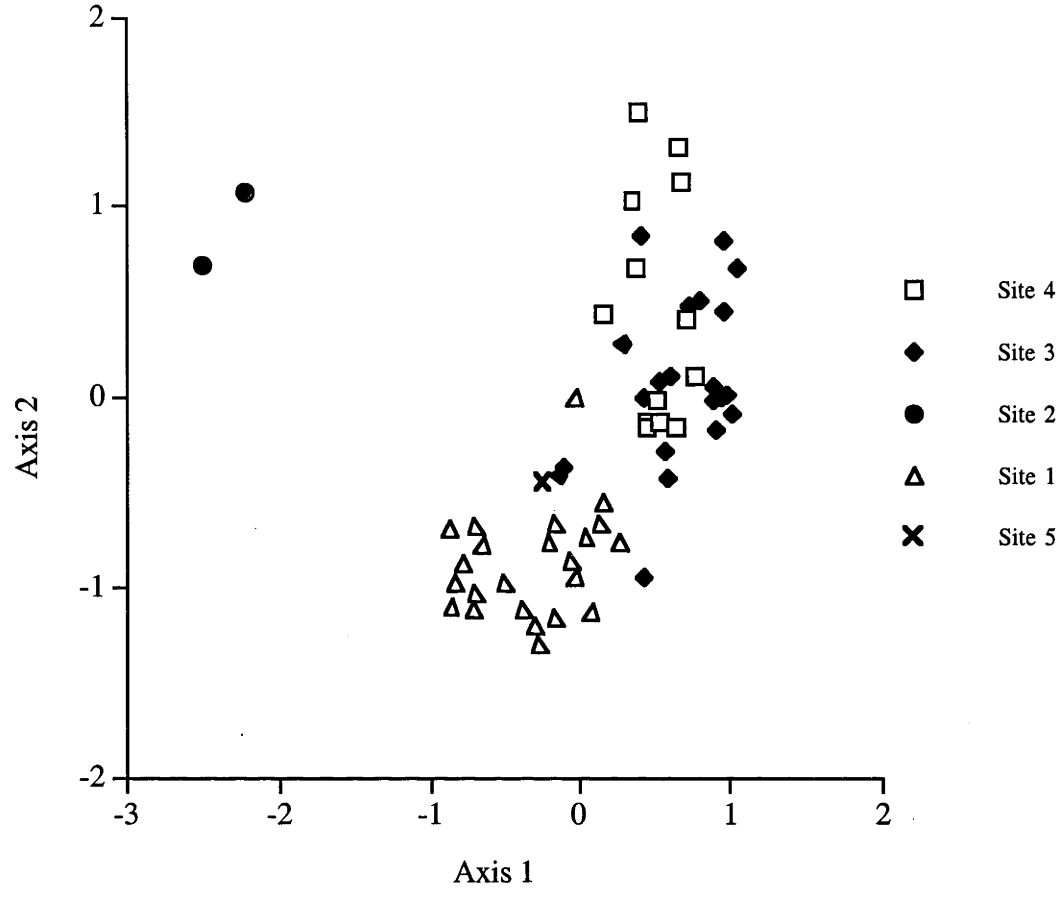

Figure 2.2 Principal coordinate analysis (PCA) of the 83 *Zieria prostrata* individuals sampled, including individuals from the four extant sites (sites 1-4) and an individual reputedly rescued from the now extinct population (site 5). Axis 1 extracted 30.02% of the variance, and axis 2 extracted 17.5% of the variance.

Table 2.4 An analysis of molecular variance (AMOVA) within and among *Zieria prostrata* populations[a] and within and among geographic regions[b]. Statistics include the degrees of freedom (DF), sums of squared deviations (SS), mean squared deviations (MS), variance component estimates, and the percentage of the total variance contributed by each component.

Source of variation	DF	SS	MS	Variance component[c]	% total variance
Among populations	2	57.8	28.9	1.12	37%
Among individuals within populations	69	132.37	1.92	1.92	63%
Among geographic regions	1	39.93	39.39	1.16	35%
Within geographic regions	70	150.79	2.15	2.15	65%

[a] Sites 1, 3 and 4.
[b] Northern, site 1 and southern, sites 3 and 4.
[c] The probability of obtaining a more extreme component estimate by chance alone is <0.0010.

2.4 Discussion

2.4.1 Population genetic structure

The four extant populations of *Zieria prostrata* were found to be highly divergent, although there was considerable overlap between sites 3 and 4. The smallest population (site 2) was the most divergent, despite being geographically intermediate between sites 1 and 3. Divergence among populations was mainly attributable to the cumulative impact of marker frequency differences among populations and for site 2, also to fixed differences. The high level of divergence among populations was unexpected given that the range of the species extends only three kilometres, and generally exceeds population divergence detected over much greater areas in several previously published genetic studies utilising RAPD markers (Table 2.5). Such extreme divergence, given the small geographic range of *Z. prostrata*, may be explained by several factors including the breeding system of the species', genetic isolation of populations and/or genetic drift.

The existence of a close association between a species breeding system and the distribution of genetic variation is well known (Hamrick and Godt 1989; Hamrick *et al.* 1991; Loveless and Hamrick 1984). The extent and distribution of allozyme diversity within plants conforms more or less to one of two common patterns. In predominant outcrossers, on average only 20% of the genetic diversity of a species is represented among populations, with the remainder existing as variation among individuals within populations. In contrast, in predominant selfers 50% of the genetic variability is found among populations (Hamrick *et al.* 1991). The level and partitioning of RAPD variation has been examined in both outcrossing and selfing plants and similar patterns as found for allozymes have been reported (Table 2.5). For example, all outcrossing species exhibit relatively low levels of variation among populations, ranging from 0% for *Banksia cuneata* to 27% for *Buchloe dactyloides*. Selfing species exhibit much greater differentiation among populations, ranging from 35% for *Cerastium fischerianum* var. *molle* to 63% for *Vicia dumetorum*. *Zieria prostrata* was found to exhibit 37% variation among populations and therefore appears to be characterised by more extensive genetic differentiation among populations than that which is typical for outcrossing species. These results may reflect greater levels of selfing than outcrossing for *Z. prostrata* and/or that mating among close relatives is occurring. Bagging experiments on plants in cultivation have revealed that *Z. prostrata* is capable of autogamy and high levels of seed set in the field, despite a lack of obvious pollinators, suggest self-pollination is likely (see Section 4.1.3.1).

Table 2.5 The level of genetic variation among populations of various plant taxa as determined by RAPD markers and AMOVA. The approximate maximum distance among populations (maximum distance) is also provided. Taxa have been sub-divided into those that are reported to be predominantly outcrossing and those that are predominantly selfing.

Species	Variance among popns. (%)	Maximum distance (kilometres)	Reference
Outcrossing:			
Banksia cuneata	0	195	Maguire and Sedgley (1997)
Buchloe dactyloides	19-27	70	Huff *et al.* (1993)
Cardamine lilacina	15	6	Nolan *et al.* (1996)
Eucalyptus amygdalina	15	?	Sale *et al.* (1996)
Eucalyptus globulis	5-26	150	Nesbitt *et al.* (1995)
Eucalyptus risdonii	8	?	Sale *et al.* (1996)
Grevillea barklyana	13	20	Hogbin *et al.* (1998)
Grevillea scapigera	13	540	Rossetto *et al.* (1995)
Hippophae rhamnoides	15	800	Bartish *et al.* (1999)
Leucadendron elimense	27	?	Tansley and Brown (2000)
Prunus africana	22-23	?	Dawson and Powell (1999)
Selfing:			
Cerastium fischerianum var. *molle*	35	?	Maki and Horie (1999)
Gentianella germanica	37	200	Fischer and Matthies (1998)
Hordeum spontaneum	43	250	Dawson *et al.* (1993)
Vicia dumetorum	63	?	Black-Samuelsson and Anderson (1997)

Genetic differentiation among *Z. prostrata* populations demonstrates genetic isolation of the populations, either current or previous. Gene flow between the headlands may be minimal or non-existent due to restricted pollen and seed dispersal. Seed dispersal experiments have shown that *Z. prostrata* seeds disperse ballistically to a maximum distance of only 1.5 m (mean ± SE = 42 ± 3.8 cm) (unpub.) and may rarely be dispersed secondarily (Section 4.4.1.2). Observations of seedling emergence in the field also support short-distance dispersal, with all observed seedlings being within 30 cm of an adult plant (pers. obs.). Further evidence for restricted gene flow, even within populations, is provided by the detection of duplicated genotypes within both sites 2 and 4. The most likely explanation for these duplicated genotypes is mating among close relatives within semi-isolated sub-populations (Section 2.3.2). The three sub-populations within site 4 are all separated by less than 50 m, implying that both pollen and seed dispersal are restricted to less than 50 m. The apparent increasing genetic

distance with geographic distance among populations of *Z. prostrata* (with the exception of site 2) also supports restricted gene flow between the headlands.

When populations are small and isolated from one another, genetic drift will also influence genetic structure and increase differentiation among populations (Barrett and Kohn 1991; Ellstrand and Elam 1993). Thus, the divergence among *Z. prostrata* populations may be further accentuated by genetic drift due to their small population size. This may explain the extreme divergence of the small population at site 2 despite it being geographically intermediate between sites 1 and 3 (Figure 1.1, Chapter 1).

2.4.2 Genotype of individual reputedly rescued from extinct population

The PCA clustering of the individual from site 5 with individuals from the extant populations (Figure 2.2) was unexpected. Given that extensive genetic divergence occurs among populations within this species across a geographic range of only three kilometres, it was expected that a population 24 kilometres south would show considerable divergence from the other populations, perhaps similar to, or greater than found for the site 2 individuals.

Initially it was thought that the genetic similarity of the site 5 individual to individuals from site 3 might be explained by a sampling or labelling error in the *ex-situ* collection. Thus, the 'source' plant growing in the botanic gardens was included in the genetic analysis, along with the reintroduced individual. Because both individuals showed the same genotype, a labelling or sampling error during the reintroduction phase could be ruled out. Prompted by this anomaly, details of the extinct population were re-investigated. Searches for herbarium and cultivation records reputedly taken prior to the population extinction were made, and those people who were associated with the original collection were interviewed. All avenues of investigation failed to provide any convincing evidence that *Z. prostrata* grew at site 5. The available evidence suggests the collection instead most likely came from site 3, but was incorrectly ascribed to site 5. Subsequent searches at site 5 failed to find any trace of the plant, probably leading to the claim of extinction, which in turn provided motivation for an entire reintroduction program. In the current recovery plan for *Z. prostrata*, it is now concluded that reports of the species occurrence at site 5 are doubtful, and the planned reintroduction program has been abandoned (NPWS 1998).

This case provides an example of how a lack of careful documentation and validation can ultimately and unintentionally lead to misinformation. Unfortunately, such lack of documentation appears to be a common problem in threatened species recovery and is

complicated by the diverse range of individuals and organisations involved in recovery efforts (Hogbin and Peakall 2000; Milton *et al.* 1999).

2.4.3 Conservation implications

The dismissal of the prior existence of the fifth population of *Zieria prostrata* (site 5), has already had direct management outcomes: the abandonment of a major reintroduction program. This represents a very significant change in the management plan for the species, with significant cost saving benefits, and emphasises the importance of using genetic studies to clarify the source of *ex-situ* collections of uncertain origin (see Section 5.3.2). Without this genetic study, further expensive and time-consuming reintroduction would have proceeded. Even so, one could argue that this genetic study was not necessary for uncovering the problem. Merely re-evaluating the evidence for the previous existence of the population would have resulted in the same outcome. Nevertheless, in this case, genetic research did provide the impetus for the re-evaluation since it raised doubt about previously assumed 'facts' that would otherwise have not been disputed. With the abandonment of any further reintroduction of site 5, managers were now concerned primarily with the management of the four extant populations.

The extensive genetic divergence detected among *Z. prostrata* populations has a number of implications for the conservation management of the extant populations. Firstly, the loss of any one population would lead to a considerable loss of genetic diversity and thus it is important that all populations be conserved. A second implication of the high divergence among populations of *Z. prostrata* is that an adequate *ex-situ* collection must sample the full range of genetic diversity from all populations. In this respect it appears that the original *ex-situ* collection was inadequate, being based on just a few individuals from two of the populations (Section 1.2). If an *ex-situ* collection is deemed necessary for the effective conservation of *Z. prostrata*, then further sampling is required. A final conservation implication of the high divergence among populations of *Z. prostrata* is that the potential genetic consequences of mixing populations may be an important conservation consideration if further population enhancement is to go ahead. As discussed in Section 2.1.2.1.2, the mixing of genetically distinct populations may give rise to outbreeding depression, whereby a reduction in fitness arises due to a loss of local adaptation or break up of co-adapted gene complexes. Therefore, if further population enhancement is to occur, it is important that only local source plants are used, as the 'precautionary principle' (Myers 1993) claims that conservation action should not be undertaken unless it can be shown not to be damaging.

2.4.4 Conclusions

The main objective of this study was to gain insight into the population genetics of *Zieria prostrata* to assist in the design of a translocation program involving reintroduction at site 5 and enhancement of the extant populations. This study, however, led to the dismissal of evidence supporting the prior existence of a *Z. prostrata* population at site 5 and resulted in the abandonment of the reintroduction program. After abandonment of reintroduction to site 5, managers were concerned primarily with the conservation of the four extant populations. The extensive differentiation detected among the four extant populations provided valuable recommendations for their management. Firstly, if further population enhancement is to occur within the extant populations, it is important that only local source plants are used given the potential for outbreeding depression. Secondly, the loss of any one population would lead to a dramatic loss of genetic variation and thus it is important that all populations be conserved. Finally, the high divergence among populations of *Z. prostrata* suggests that an adequate *ex-situ* collection must sample the full range of genetic diversity from all populations.

CHAPTER 3

Relationship of *Zieria prostrata* to the widespread *Z. smithii* species complex

3.1 Introduction

3.1.1 Background

In the previous chapter, the genetic relationship among the four extant populations of *Zieria prostrata* was outlined. How best to preserve the genetic diversity present within this highly restricted species was also discussed. However, the discovery of another headland *Zieria* taxon, very similar to *Z. prostrata*, cast doubt upon both the taxonomy and distribution of *Z. prostrata*. After extensive survey, the newly discovered headland *Zieria* taxon, hereafter referred to as *Z.* sp. aff. *smithii,* was found to occur at ten coastal sites along approximately 600 kilometres of coastline, south of the *Z. prostrata* populations (Hogbin 1999). Although *Z. prostrata* is widely accepted, and referred to, as a distinct species, a formal description has not yet been published (Section 1.2). Prior to undertaking any further management of *Z. prostrata*, it was vital that the relationship of *Z. prostrata* to the newly discovered headland taxon be investigated. Given that considerable conservation resources are devoted to the management of threatened species, commensurate attention should be devoted to ensuring the adequacy of a species description (Avise 1989).

Taxonomic clarification is especially vital for threatened plant species, as decisions regarding the delineation of species have important implications for conservation and management (Hibbett and Donoghue 1996; Hopper 1993; Shapcott 1998; Soltis and Gitzendanner 1999). Inappropriate taxonomy may lead to a failure to recognise and thus conserve threatened species. For example, a taxonomic revision of what was thought to be the single species *Eucalyptus redunca* (Brooker and Hopper 1991) recognised 27 species. A few of these 27 segregate species are highly localised and endangered. Without such taxonomic clarification, these newly recognised endangered species warranting immediate management attention would have gone unrecognised (Hopper 1993). Such failure to recognise species not only impinges upon the conservation of the species concerned, but also upon the conservation status of the community and thus reserve acquisition (Hopper 1993; Rojas 1992; Shapcott 1998). Alternatively, inappropriate taxonomy may lead to greater priority being placed upon a taxon than deserved. Given the reality of meagre resources available for conserving a multitude of threatened species, it is important to ensure funds are directed to the most appropriate taxa. For example, the application of species status to a group of populations that

merely form part of a more widespread species could lead to greater emphasis being placed upon preserving the group than may be necessary or justified.

3.1.2 Current taxonomy of *Zieria prostrata* and *Z. smithii*

Within the genus, *Zieria prostrata* belongs to the *smithii* group, which comprises three other species: *Z. montana, Z. robertsii* and *Z. smithii* (Armstrong ms). Of these species, *Z. prostrata* is most closely related to *Z. smithii* (Armstrong ms). *Zieria smithii* occurs along the eastern coast of Australia from north-east Queensland to north-east Victoria and comprises two subspecies: subsp. *smithii* and subsp. *tomentosa* (Armstrong ms). A formal description of these two subspecies has not yet been published, and they are currently recognised as subsp. A and subsp. B respectively (Armstrong 1991). All *Z. smithii* populations included within this study were subsp. *smithii*, as subsp. *tomentosa* occurs from Northern NSW to north-east Queensland, north of the study area.

Zieria smithii is the most common of the 42 *Zieria* species (Armstrong ms) and exhibits considerable morphological variation throughout its range. Additionally, casual observation of *Zieria* herbarium specimens lodged at the National Herbarium of NSW revealed a large number of unidentified *Zieria* specimens tentatively identified as *Z.* sp. aff. *smithii*. It is obvious that *Z. smithii* is a variable species complex. Species complexes such as this may arise through for example; ecological clines, hybrid zones, or active speciation. Alternatively, a species complex may be an artefact of a lack of taxonomic study of the group in question, resulting in a number of taxa being grouped together under one name.

The main distinguishing characters between *Z. smithii* and *Z. prostrata*, based on descriptions in Armstrong (ms), are summarised in Table 3.1. Briefly, *Z. smithii* is an erect to robust shrub to 2 m in height, which occurs on rainforest margins or in wet sclerophyll forest. In contrast, *Z. prostrata* is restricted to grassy headlands where it grows as a prostrate or low sprawling shrub to less than 0.5 m in height. *Zieria smithii* can be distinguished from *Z. prostrata* by its narrow elliptic central leaflet and warted branches lacking ridges, compared with the oblong central leaflet and bare ridged branches of *Z. prostrata.*

Zieria sp. aff. *smithii* is similar to *Z. prostrata* in that it also possesses a prostrate or low sprawling habit and grows upon headlands. On the other hand, *Z.* sp. aff. *smithii* is similar to *Z. smithii* in that its stems have small warts and lack ridges. Leaf morphology of *Z.* sp. aff. *smithii* appears intermediate between that of *Z. prostrata* and *Z. smithii*. However, these simple distinctions are not always clear cut, as both *Z.* sp. aff. *smithii*

and *Z. smithii* display considerable morphological variation in both leaf shape and wart density.

Table 3.1 The main distinguishing characters between *Zieria smithii* and *Zieria prostrata,* as described by Armstrong (ms.).

Character	*Zieria prostrata*	*Zieria smithii*
Habit	Prostrate shrub, forming mats up to 0.5 m in diameter.	Erect robust shrub to 2 m.
Branches	Prominently ridged, not tuberculate and glabrous.	Slightly ridged when young, tuberculate all over, and either glabrous or subglabrous to tomentose all over with short stellate hairs.
Petiole	c. 3-4 mm in length and not tuberculate.	To 16 mm in length and tuberculate (± strongly so).
Central leaflet	Obovate in shape, 10-16 mm long, 4-6 mm broad, apex obtuse/rounded, not discolourous, not tuberculate, and glabrous.	Narrow elliptic, oblong or lanceolate in shape, to 45 mm long, 7 mm broad, apex acute, darker on upper surface, tuberculate and glabrous or hirsute to tomentose with scattered stellate hairs throughout.

Given the discovery of the headland *Z.* sp. aff. *smithii*, the considerable morphological variation apparent within *Z. smithii*, and the lack of a published description of *Z. prostrata*, it was vital the relationship between *Z. prostrata* and these taxa be investigated. It is worth noting here that the description of *Z. prostrata* was apparently made on the basis of a single specimen collected from a single population within the species range (Armstrong ms). Of particular interest was whether the phenotypic differences observed between the headland and inland populations reflect genetic differences, or are plastic and environmentally influenced. For example, many coastal ecotypes are prostrate or procumbent, a habit considered adaptive to frequent high winds and salt spray near coastal sites, whereas the inland populations are erect (Gottlieb 1984).

There are three alternative hypotheses for the taxonomic status of *Z. prostrata* and each has different conservation implications. Firstly, *Z. prostrata* may be taxonomically distinct from both *Z. smithii* and *Zieria* sp. aff. *smithii*. In this scenario, the current management of *Z. prostrata* would not change. Secondly, *Z. prostrata* may be taxonomically distinct from *Z. smithii*, but not from *Z.* sp. aff. *smithii*. In this scenario,

the geographic distribution of *Z. prostrata* would greatly expand from only three kilometres to over 600 kilometres. This would require an expansion of management efforts to cover the management needs of the additional populations. Indeed, given the increased geographic range, this outcome would likely change the conservation status of *Z. prostrata* from endangered to vulnerable, thus also altering the species management priority. Finally, *Z. prostrata* and *Z.* sp. aff. *smithii* may merely be headland ecotypes of *Z. smithii.* In this scenario *Z. prostrata* would no longer be worthy of specific status and its priority for conservation management may be greatly reduced.

3.1.3 Species recognition

3.1.3.1 Species concepts

So how do we determine whether the group of populations, now recognised as *Z. prostrata*, should be considered a distinct species? In an attempt to answer this sort of question, systematists and evolutionary biologists have come up with an increasingly diverse array of species concepts (see reviews by Davis 1997; de Queiroz 1998; Goldstein *et al.* 2000; Harrison 1998; Soltis and Gitzendanner 1999). However, not one of these concepts has been universally accepted, leading to what has become known as 'the species problem'. However, de Queiroz (1998) recently proposed that all modern species concepts are merely variations of the same general species concept. He proposes 'all modern species definitions either explicitly or implicitly equate species with segments of population level evolutionary lineages' and refers to this sentiment as 'the general lineage concept of species.' Therefore, all species concepts, despite their diversity, aim to recognise distinct lineages: single lines of direct ancestry and descent.

When it comes to clarifying the taxonomy of a taxon for conservation purposes, irrespective of the species concept applied, it is vital that recognised 'species' do indeed represent distinct evolutionary lineages, as a failure to do so can have a considerable impact upon effective conservation (Avise 1989; Goldstein *et al.* 2000; Hibbett and Donoghue 1996; Rojas 1992; Soltis and Gitzendanner 1999). Species concepts that incorporate history and reflect phylogeny will ultimately be more useful for preserving biodiversity than those that do not (Hibbett and Donoghue 1996; Soltis and Gitzendanner 1999). The application of a species name to something other than a phylogenetic unit can thwart conservation efforts by failing to detect, and thus protect, the real genetic diversity represented within the species (Hibbett and Donoghue 1996; Rojas 1992; Soltis and Gitzendanner 1999). For example, conservation of a supraspecific group that includes more than one phylogenetic species, or of a subspecific group that represents only a portion of an interacting assembly of

individuals or populations, may not have the desired effect of preserving the biological units that warrant saving (Soltis and Gitzendanner 1999).

Given the concerns outlined above, and that conservation is fundamentally concerned with conserving evolutionary lineages, the phylogenetic species concept is particularly favoured within the conservation literature (Goldstein *et al.* 2000; Hibbett and Donoghue 1996). The phylogenetic species concept is a term which has been used to describe at least three distinct classes of species definitions (e.g. Cracraft 1983; Donoghue 1985; Panchen 1992), however they are all similar in that they focus upon the historical relatedness of organisms and the distribution of characters (Baum 1992). Indeed, de Queiroz (1998) claims the term 'phylogenetic species concept' should not be restricted to the above mentioned species definitions, as it is a term that accurately describes all modern species definitions. Despite not adhering to any specific phylogenetic species concept, within this study I attempt to recognise phylogenetic species, or in other words, distinct evolutionary lineages.

3.1.3.2 Operational species definition

In this study, an 'operational definition' (see Crisp and Weston 1993) is used, which can be equated to the 'species criteria' defined by de Queiroz (1998) as a standard for judging whether a particular entity is or is not a species. A particular entity is recognised as a species when it forms a distinct and discrete unit in phenetic space, and as a subspecies when it forms a distinct, but not discrete unit. This operational definition is superficially no different to the phenetic species concept as defined by Michener (1970): 'a species is a group of organisms not itself divisible by phenetic gaps..., but separated by such phenetic gaps from other such groups.' However, as outlined below (Section 3.1.3.3), in this study genetic markers are used to confirm that phenetic relationships based upon morphometric analysis correspond to phylogenetic relationships and thus that any discrete phenetic groups correspond to distinct evolutionary lineages (phylogenetic species).

3.1.3.3 Molecular versus morphological characters

The characters selected as indicators of evolutionary history can influence whether a distinct and discrete unit in phenetic space corresponds to a phylogenetic species. Morphological markers are the primary source of species definition in plants, as they are the most accessible source of data about evolutionary relationships (Judd 1999). However, taxonomies based on morphological characters alone sometimes provide an inadequate or misleading representation of the evolutionary history of a group of taxa, particularly at the subspecies and species levels (Avise 1989; Hillis 1987; Hopper 1993; Sytsma 1990). These inadequate or misleading representations may be due to

phenomena such as phenotypic plasticity, introgression, convergence or parallelism, and active or rapid speciation (Schaal *et al.* 1991; Sivarajan and Robson 1991; Sytsma 1990). Given these problems potentially associated with morphological data, genetic markers are increasingly being used, particularly in conjunction with morphological markers, or as a tool to test a morphological species (Avise 1989; Crawford 2000; Goldstein *et al.* 2000; Harrison *et al.* 1997; Hillis 1987; Sytsma 1990). Given the morphological complexity apparent within the *Zieria smithii* species complex and the potential for phenotypic plasticity (see Section 3.1.2), in this study morphometric analysis was used as the primary determinant of phenetic relatedness and genetic markers were then used to test that distinct and discrete units in phenetic space represented distinct evolutionary lineages.

3.1.4 Chapter objectives

The main objective of this study was to clarify the taxonomic status of *Zieria prostrata.* This was achieved through analysing both morphological and genetic variation among populations of *Zieria prostrata* and the widespread *Z. smithii* species complex, including headland populations of *Z.* sp. aff. *smithii.* A multivariate morphometric investigation was used as the primary tool for elucidating taxonomic relationships. A preliminary genetic study was used to investigate the validity of any distinct evolutionary lineages revealed by the morphometric analysis.

3.2 Methods

3.2.1 Sampling strategy

In May of 1999, a total of 23 sites were sampled, including ten populations of *Zieria* sp. aff. *smithii*, four of *Z. prostrata* and nine of *Z. smithii* (Table 3.2). A schematic representation of the sampling strategy is provided in Figure 3.1. All headland *Zieria* populations not already recognised as *Z. prostrata* were classed as *Z.* sp. aff. *smithii.* Aside from one exception, a population within Bongil Bongil National Park, any inland *Z. smithii* population was classed as *Z. smithii.* The *Z.* sp. aff. *smithii* population within Bongil Bongil National Park (Figure 3.1) occurs on an escarpment overlooking the ocean. This escarpment is now approximately four kilometres from the ocean, but was likely to be a headland less than 1.8 million years ago. This population is now within typical sheltered *Z. smithii* habitat, but plants have retained the low sprawling-prostrate habit and leaf shape typical of the headland *Z.* sp. aff. *smithii.*

Five individuals, or less when population size was smaller, were sampled from each population (Table 3.2). A total of 102 individuals were sampled. Material was collected for both morphometric and genetic analysis from each plant sampled. In addition,

herbarium specimens were collected from each population and lodged at the National Herbarium of NSW, Sydney. Material for genetic analysis was placed into liquid nitrogen soon after collection. Material for morphometric analysis was pressed. The height and habit (prostrate, sprawling, or upright) of each individual was also recorded. Given that sampling covered a geographic range of approximately 600 kilometres, not all populations were flowering during sampling and consequently floral characters were not included in the morphometric analysis.

Table 3.2 An outline of the sites sampled for morphometric and genetic analysis. All sampled individuals were included in the morphometric analysis, however not all were included in the genetic analysis (see Section 3.2.3.1 for reasons). When not all sampled individuals were included in the genetic analysis, the number included is provided in brackets.

Population	Taxon	No. individuals sampled	Latitude and Longitude
Site 4	*Z. prostrata*	5	30°10'44"S 153°11'08"E
Site 3	*Z. prostrata*	5	30°10'28"S 153°11'19"E
Site 2	*Z. prostrata*	2	30°10'00"S 153°11'31"E
Site 1	*Z. prostrata*	5	30°09'30"S 153°12'02"E
Boomerang Point	*Z.* sp. aff. *smithii*	5 (3)	30°20'58"S 152°32'24"E
Diamond Head	*Z.* sp. aff. *smithii*	8	31°44'00"S 152°50'20"E
Little Grants Head	*Z.* sp. aff. *smithii*	5	31°36'35"S 152°50"35"E
Nobby Head	*Z.* sp. aff. *smithii*	5 (4)	31°26'58"S 152°56'10"E
Big Hill Point	*Z.* sp. aff. *smithii*	5	31°17'13"S 152°58'01"E
South of Hat Head	*Z.* sp. aff. *smithii*	2	31°03'52"S 153°03'28"E
Hat Head	*Z.* sp. aff. *smithii*	5 (4)	31°03'37"S 153°03'34"E
Valla Headland	*Z.* sp. aff. *smithii*	3	30°35'36"S 153°00'45"E
Bongil Bongil	*Z.* sp. aff. *smithii*	5	30°26'51"S 153°03'23"E
Diggers Head	*Z.* sp. aff. *smithii*	1	30°16'55"S 153°08'39"E
Sandy Beach	*Z. smithii*	3	30°09'42"S 153°11'19"E
Beacon Hill	*Z. smithii*	3	30°18'44"S 153°08'03"E
Coffs Harbour SF	*Z. smithii*	5	30°10'04"S 153°08'43"E
Kempsey	*Z. smithii*	5 (4)	31°04'56"S 152°38'37"E
Bellangry SF	*Z. smithii*	5 (0)	31°17'16"S 152°33'05"E
Alum Mountain	*Z. smithii*	5 (2)	32°32'34"S 152°18'42"E
Mungo Brush	*Z. smithii*	5 (2)	32°25'00"S 152°13'15"E
Kylies Hut	*Z. smithii*	5 (4)	31°44'10"S 152°47'25"E
Grants	*Z. smithii*	5	30°36'35"S 152°50'13"E

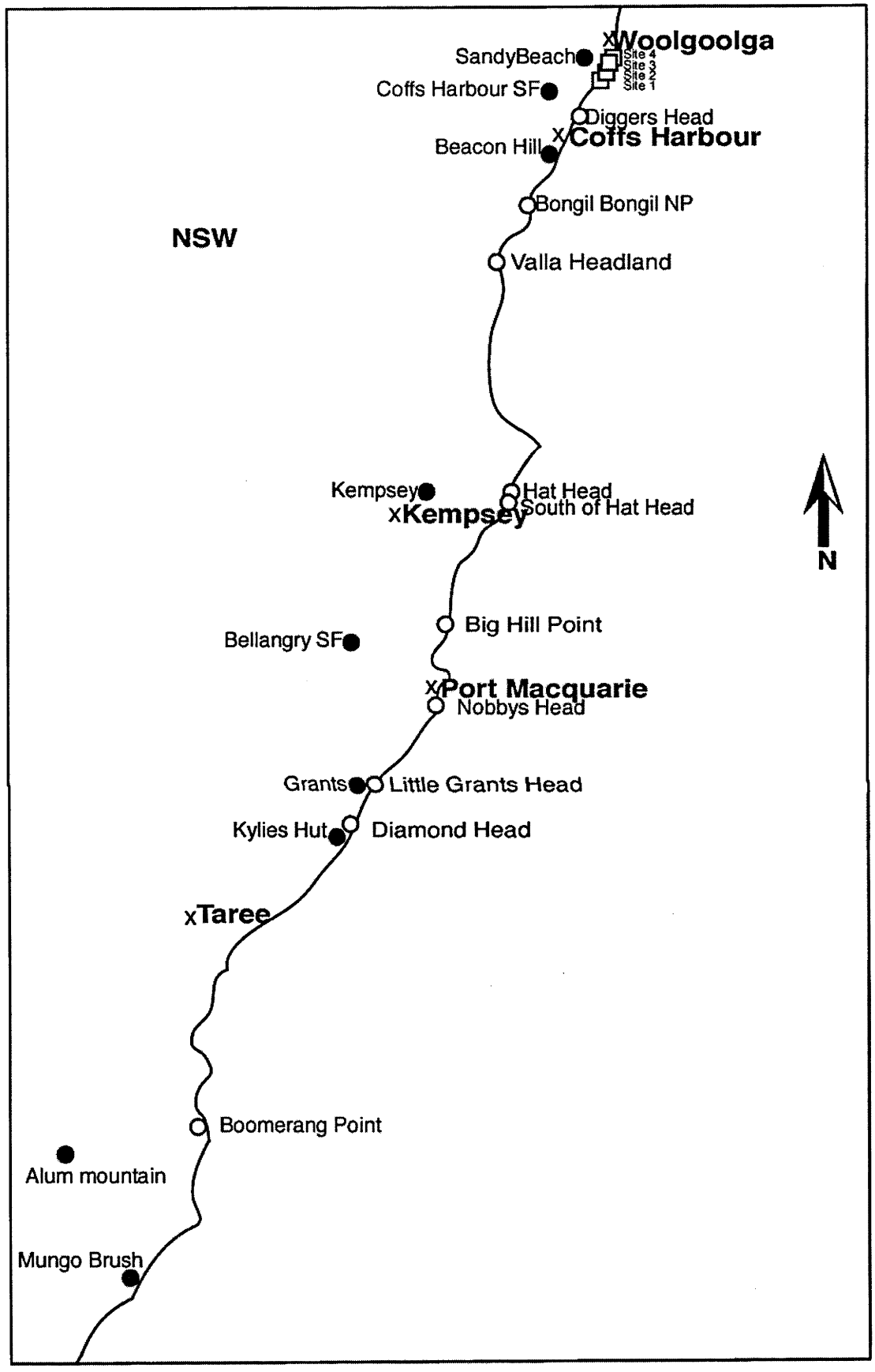

Figure 3.1 Schematic representation of the sampling strategy for morphometric and genetic analysis, depicting the various population collection sites for *Zieria prostrata* (□), *Z. smithii* (●) and Z. sp. aff. *smithii* (○).

3.2.2 Morphometric analysis

3.2.2.1 Character measurement

A total of 13 characters were scored for all 102 individuals (Table 3.3). Two characters were categorical variables, 11 were continuous variables, and two of the continuous characters were ratios. All the measurements were made in millimetres. The data for all categories, except the two categorical characters (habit and wart prominence) were log-transformed. The majority of characters are self explanatory, however a brief outline of those not so obvious follows.

Habit was assigned as one of three categories: prostrate (0), sprawling (1) and upright (2). Central leaf shape was determined by measuring the distance from the leaf tip to the widest point, and dividing this by the leaf width. For all the density characters (e.g. leaf oil gland density and stem wart density), the number of glands, hairs or warts was measured for a circular area of 4 mm in diameter at the centre of the leaf or stem. For wart prominence, three categories were assigned: warts rare or inconspicuous (0), warts obvious (1) and warts prominent (2). For ridge presence, ten nodes of each individual were scored for ridge presence, and then the number of ridges was divided by ten to provide a ratio. For all stem characters (excluding inter leaf length), measurements were taken at the third internode from new growth to standardise age. Each character was scored up to ten times for each individual (less when the specimen did not provide enough material) and the mean value was used for subsequent analyses.

Table 3.3 Morphometric characters measured, where # denotes a categorical variable (all others continuous), * indicates a ratio and @ denotes character data that has been log-transformed.

Character
Habit#
Leaf characters
Central leaf length@
Leaf shape (length to widest point/width) *@
Petiole length@
Oil gland density@
Hair density-lower surface@
Hair density-midvein@
Hair density-upper leaf surface@
Wart prominance#
Stem characters
Inter leaf length@
Wart density@
Hair density@
Ridge presence*@

3.2.2.2 Analysis

The phenetic methods of ordination and cluster analysis were used to analyse the morphological characters. Both methods group individuals based on their similarity, but differ slightly in the manner of visualising these groups. An overview of these approaches is provided by Crisp and Weston (1993), James and McCulloch (1990), Pimentel (1981), Reyment *et al.* (1984) and Thorpe (1983). Briefly, ordination techniques represent the relationships of individuals by the scattering of points in two or more dimensions, and thus allow the identification of non-hierarchical and multiple overlapping patterns (Faith and Norris 1989; Sneath and Sokal 1973), whereas cluster analysis imposes a hierarchical structure upon the data irrespective of whether this structure exists or not (Thorpe 1983). Given that phenotypic characters are unlikely to be distributed among populations in a hierarchical manner (Thorpe 1976), ordination is considered superior to cluster analysis when undertaking population level studies (Crisp and Weston 1993). However, given the value of applying a variety of multivariate techniques in exploratory analysis at low taxonomic levels (Crisp and Weston 1993), both cluster and ordination analyses were performed. Analyses were performed using the Pattern Analysis Package (PATN) (Belbin 1989). The Gower metric (range-standardised Manhattan) distance measure was used to calculate a matrix of distances between all individuals, and this distance matrix subsequently formed the input for both ordination and cluster analysis.

3.2.2.2.1 Ordination

Both metric multidimensional scaling (MMDS) and non-metric multidimensional scaling (NMDS) were used. Both methods gave similar results so only the NMDS results have been presented. Similarly, all analyses were performed in both two and three dimensions. In all cases the third dimension added little information and therefore only the results for two dimensional NMDS have been presented.

3.2.2.2.2 Cluster analysis

The unweighted pair-group method using arithmetic averages (UPGMA) (Sneath and Sokal 1973) method of cluster analysis was used. The relative contribution of each character in determining relationships in the cluster analysis was determined by their Cramer value. Cramer values, calculated as the between group variance divided by total variance, estimate the contribution made by any character to the classification (Belbin 1989). Cramer values vary from 0 (no difference between groups) to 1 (perfect discrimination between groups). Characters with Cramer values of less than 0.7 were not considered to contribute significantly to classification relationships.

3.2.3 Genetic analysis

Randomly Amplified Polymorphic DNA (RAPD) markers were chosen for this study because they were known to be reproducible and informative in *Zieria prostrata* (Chapter 2). RAPD markers have been used extensively to clarify systematic relationships among closely related taxa (e.g. Brunell and Whitkus 1997; Coleman *et al.* 2000; Demeke *et al.* 1992; Harrison *et al.* 1997; James and Brown 2000; Sale *et al.* 1996; Smith *et al.* 1996; Stewart *et al.* 1996; Van Bruren *et al.* 1994). Nevertheless, one potential limitation of using RAPD data for systematic analysis is the potential for co-migration of non-homologous bands (Hurme and Savolainen 1999; Quiros *et al.* 1995; Whitkus *et al.* 1994; Williams *et al.* 1993). Fortunately, studies have shown that among closely related taxa, RAPD bands are generally homologous, with the incidence of non-homology increasing with taxonomic distance (Brauner *et al.* 1992; Rieseberg 1996).

3.2.3.1 DNA extraction

DNA was initially isolated from frozen leaf tissue following the procedure outlined in Section 2.2.2. DNA was successfully isolated from the majority of individuals, however, the DNA of all the *Zieria* sp. aff. *smithii* individuals and most of the *Z. smithii* individuals could not be successfully amplified (during the RAPD-PCR process outlined in Section 3.2.3.2). This amplification failure was presumably a consequence of the presence of an inhibitory compound within the leaves which was co-extracted during the DNA extraction process. Interestingly, this inhibitory compound was apparently not present in any of the *Z. prostrata* populations or in the two nearby populations of *Zieria smithii* (Sandy Beach and Coffs Harbour State Forest).

The use of Microcon® filters (Microcon YM-100; Millipore) was found to remove the PCR inhibitor from many, but not all, samples. For the remaining individuals, DNA was re-extracted from the frozen leaf tissue using an extraction technique described by Jobes *et al.* (1995). This technique successfully increased the number of amplifiable samples, though some still failed to amplify and were excluded from the study. Therefore, only 79 of the 102 individuals included in the morphometric analysis were also included in the genetic analysis (Table 3.2).

3.2.3.2 PCR-RAPD procedure

The RAPD-PCR conditions were as outlined in Section 2.2.3. In an initial survey, 100 decamer primers (Operon Technologies Inc, California. Kits: OPA, OPB, OPK, OPAA and OPAB) were evaluated for suitability. One *Z. prostrata* individual was screened with all 100 primers. Of these 100 primers, 47 showed potentially useful profiles. To

screen for polymorphisms, these 47 primers were screened further with one individual from each of the three taxa. Four primers (OPA 20, OPK 4, OPAB 5, and OPAB 18) were selected to generate RAPD profiles for all 79 individuals.

The resolution and scoring of amplification products was undertaken as outlined in Section 2.2.3. However, in this study, the RAPD profiles were scored in a highly conservative way. Each primer revealed 10-20 polymorphic bands, however not all were scored. Given time limitations, only 3-5 of the most prominent polymorphic bands per primer were scored. A total of 13 strong and reproducible polymorphic bands were scored.

3.2.3.3 Data analysis

The genetic data was analysed in the same manner as the morphometric data, following the methods outlined in Section 3.2.2.2. Briefly, a genetic distance matrix was calculated using the Gower metric and this distance matrix subsequently formed the input for both two dimensional NMDS and UPGMA cluster analysis.

Additionally, the genetic and morphological data were combined into a single data set, comprising the 13 morphological characters and the 13 genetic characters. This data set contained only the 79 individuals for which both genetic and morphological information was available, and was analysed as outlined in Section 3.2.2.2. Briefly, a distance matrix was calculated for the combined genetic and morphological data set using the Gower metric. This distance matrix was then used as input for both two dimensional NMDS and UPGMA cluster analysis. Only results of the two dimensional NMDS are depicted given that both techniques revealed similar outcomes.

3.3 Results

3.3.1 Morphometric analysis

The NMDS ordination of the morphometric data is presented in Figure 3.2. *Zieria prostrata* clearly forms a separate group in the ordination space, distinct and discrete from *Z.* sp. aff. *smithii* and *Z. smithii.* For *Z. smithii* and *Z.* sp. aff. *smithii*, there is obvious grouping of individuals into two clusters, however there is overlap between them. The overlapping is predominantly due to the Alum Mountain and Bellangry State Forest populations of *Z. smithii* grouping among *Z.* sp. aff. *smithii* individuals. Additionally, the single Diggers Head *Z.* sp. aff. *smithii* individual groups with *Z. smithii.*

There is inherent danger in including categorical data together with continuous data, where the categorical data could assign individuals to groups on the basis of those characters alone. The above analyses were therefore re-run after excluding the two categorical variables (habit and leaf wart prominence) to ensure these characters were not over assigning individuals to groups. This exclusion of habit also avoided any bias that might be introduced if habit is environmentally influenced (Section 3.1.2). The ordination of the data set after excluding habit and leaf wart prominence is shown in Figure 3.3. Even with its most distinguishing character, prostrate habit, excluded, *Z. prostrata* still groups separately from *Z.* sp. aff. *smithii* and *Z. smithii. Zieria* sp. aff. *smithii* and *Z. smithii* again form adjacent groups, with the Alum Mountain and Bellangry State Forest *Z. smithii* populations grouping with *Z.* sp. aff. *smithii*, and the Diggers Head population of *Z.* sp. aff. smithii grouping with *Z. smithii.*

The dendogram resulting from cluster analysis (Figure 3.4) revealed a similar result to the ordination. Three main groups were revealed. The first and most distant comprises only *Z. prostrata*, the second comprises predominantly *Z.* sp. aff. *smithii* and the third comprises predominantly *Z. smithii.* Again, the Alum Mountain and Bellangry State Forest populations of *Z. smithii* group partly with the *Z.* sp. aff. *smithii* individuals and the *Z.* sp. aff. *smithii* Diggers Head population clusters within the *Z. smithii* group.

The Cramer values based on the three groups in the cluster analysis (Figure 3.4), are provided in Table 3.4. All characters, except leaf oil gland density and leaf wart prominence, varied significantly among the three groups. Table 3.5 depicts the mean value of each character that varied significantly among groups. *Zieria prostrata* can be distinguished from the other two groups by its prostrate habit, shorter central leaf length, shorter petiole length, lower hair density on both leaves and stems, shorter inter leaf length, lower density of stem warts, and the occurrence of stem ridges. The

majority of *Z.* sp. aff. *smithii* characters appear to be intermediate between *Z. prostrata* and *Z. smithii.*

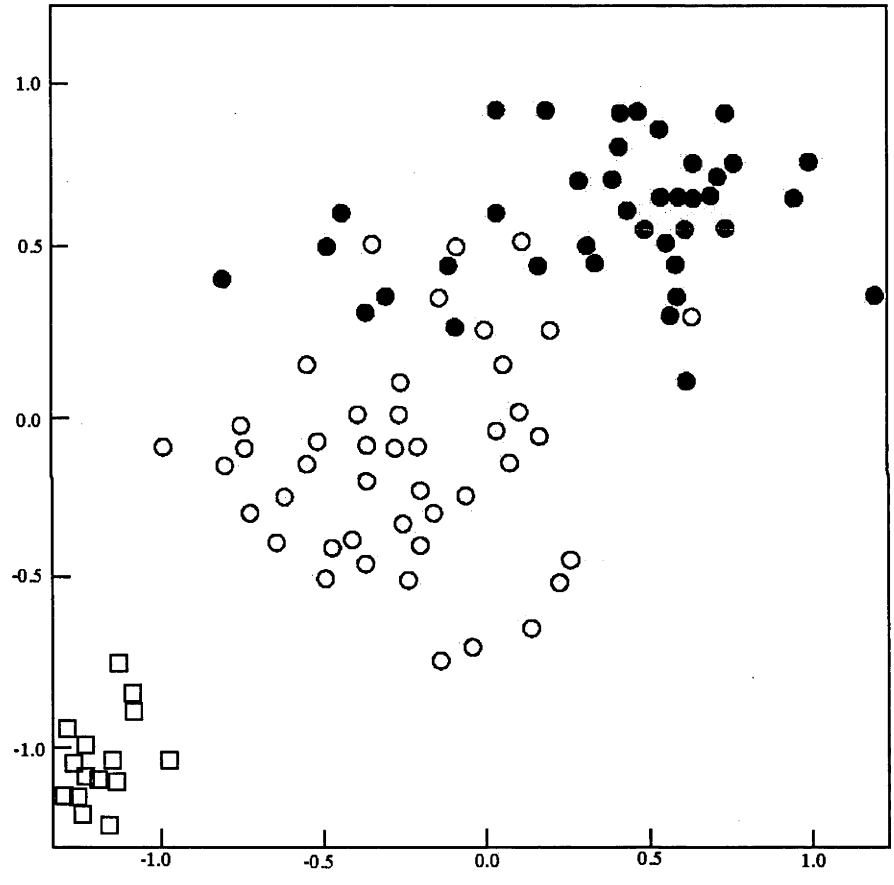

Figure 3.2 Two dimensional ordination (NMDS) of morphometric data for *Zieria prostrata* (□), *Z. smithii* (●) and *Z.* sp. aff. *smithii* (○). Stress = 0.102.

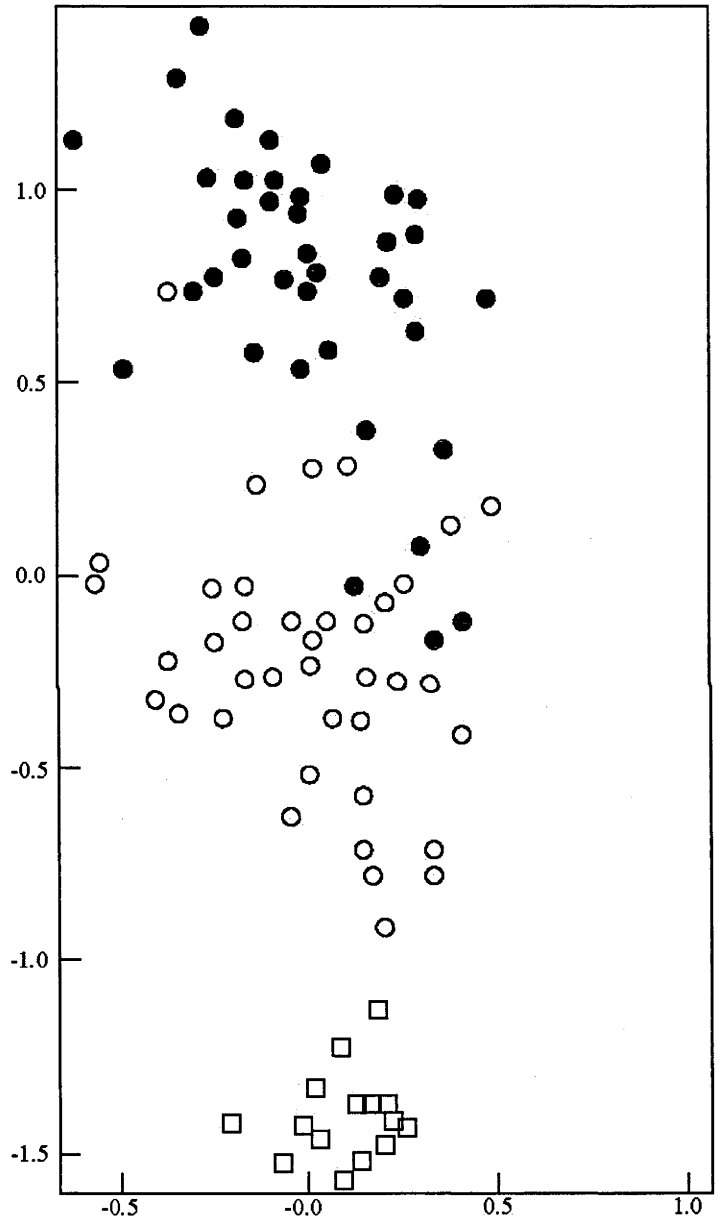

Figure 3.3 Two dimensional ordination (NMDS) of morphometric data for *Zieria prostrata* (□), *Z. smithii* (●) and *Z.* sp. aff. *smithii* (○) with the exclusion of the categorical values habit and leaf wart prominence. Stress = 0.09.

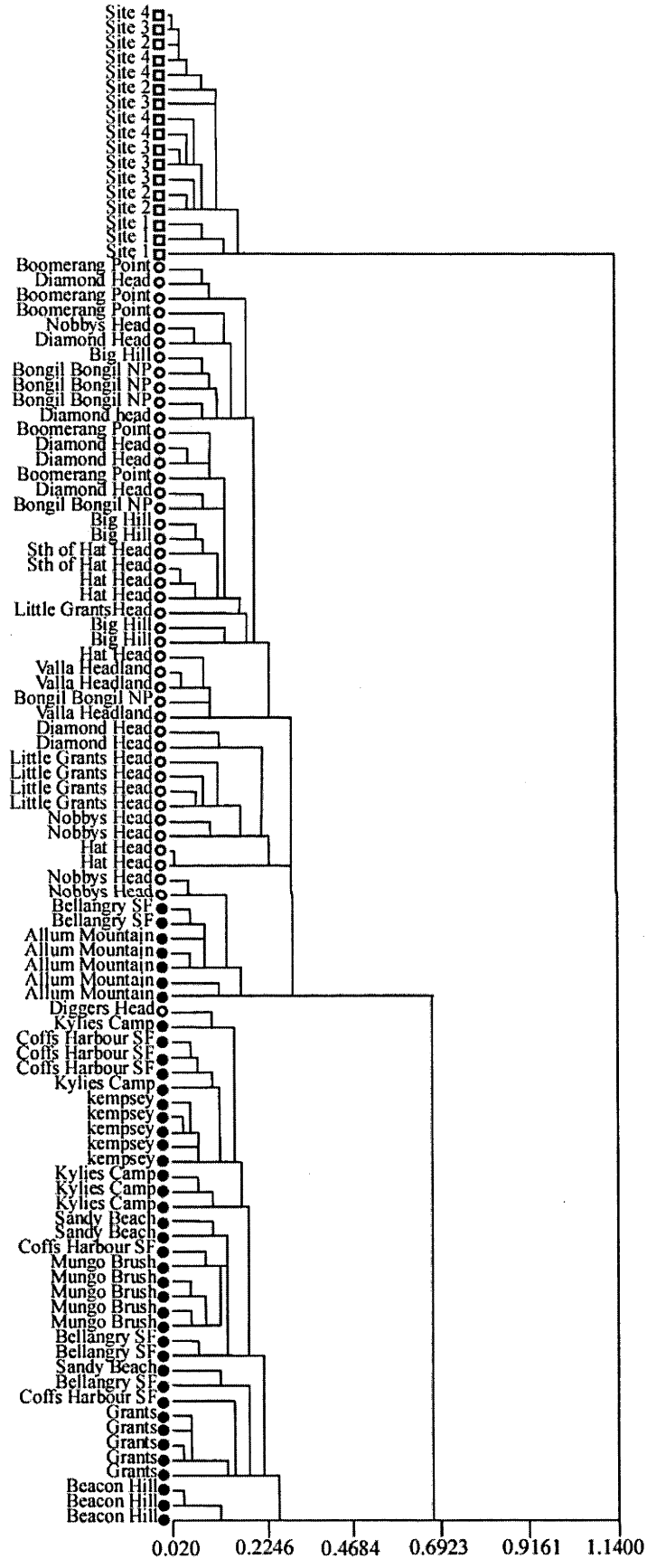

Figure 3.4 Dendogram from UPGMA cluster analysis of morphometric data for *Zieria prostrata* (□), *Z. smithii* (●) and *Z.* sp. aff. *smithii* (○).

Table 3.4 Cramer values for morphometric analysis using the three groups assigned by UPGMA cluster analysis (see Figure 3.4). A character was considered significant if the Cramer value was greater than 0.7.

Character	Cramer value
Habit	0.8145
Central leaf length	0.8886
Leaf shape	0.7278
Petiole length	0.8160
Oil gland density	0.3521
Hair density-leaf lower surface	0.8586
Hair density-leaf midvein	0.8399
Hair density-upper leaf surface	0.7805
Wart prominence-leaf	0.6601
Inter leaf length	0.7928
Wart density-stem	0.8100
Hair density-stem	0.7741
Ridge presence	0.7590

Table 3.5 Mean value (±SE) for morphological characters that varied significantly among the three taxa; *Zieria prostrata, Z.* sp. aff. *smithii* and *Z. smithii.*

Character	Mean value (±SE)		
	Zieria prostrata	*Zieria* sp. aff. *smithii*	*Zieria smithii*
Habit	0 ± 0	1.0 ± 0.03	1.9 ± 0.01
Central leaf length	15.0 ± 0.30	27.0 ± 0.40	41 ± 0.70
Leaf shape	2.5 ± 0.04	3.3 ± 0.03	4.5 ± 0.09
Petiole length	4.4 ± 0.09	7.8 ± 0.34	12.7 ± 0.17
Hair density-leaf lower surface	0.01 ± 0.01	6.8 ± 0.75	57.2 ± 2.3
Hair density-leaf midvein	0.03 ± 0.01	5.4 ± 0.28	13 ± 0.46
Hair density-upper leaf surface	0.09 ± 0.04	1.0 ± 0.09	9.2 ± 0.58
Inter leaf length	13.9 ± 5.19	17.3 ± 0.32	33.8 ± 0.59
Wart density-stem	3.1 ± 0.43	29.0 ± 0.52	23.02 ± 0.44
Hair density-stem	0.3 ± 0.05	44.6 ± 3.36	122.6 ± 7.30
Ridge presence	0.76 ± 0.01	0.22 ± 0.01	0.09 ± 0.00

3.3.2 Genetic analysis

The genetic data failed to distinguish all individuals (Figure 3.5). As outlined in Section 2.2.4, duplicated multilocus genotypes are expected within a given data set when the genetic markers fail to provide sufficient resolution, or when plants are unexpectedly large, clonal or exhibit high levels of selfing. In this instance, the duplicated genotypes are obviously a consequence of insufficient resolution given that individuals in separate populations share the same genotype. This low resolution reflects the conservative scoring of RAPD profiles (Section 3.2.3.2). Nonetheless, this low resolution is not of major concern given that markers selected for their information value at a taxonomic level will not necessarily be informative at the individual level.

The genetic data provided new information not provided by the morphological data. Most notably, there was considerable overlap between *Z.* sp. aff. *smithii* and *Z. smithii* (Figure 3.5). *Zieria prostrata,* in contrast, clusters within a single group as in the morphometric analysis. However, some individuals from the nearby inland populations of *Z. smithii* were clustered within the *Z. prostrata* group (because their genotype could not be distinguished from that of some *Z. prostrata* individuals based upon the small set of genetic markers). This trend of headland populations being genetically similar to nearby *Z. smithii* populations also appears in *Z.* sp. aff. *smithii.* For example, the Kylies Camp population of *Z. smithii* and the Diamond Head population of *Z.* sp. aff. *smithii* share the same genotype and are separated in the field by less than one kilometre. Additionally, the Grants Head population of *Z. smithii* and the Little Grants Head population of *Z.* sp. aff. *smithii* cluster together and are also separated in the field by less than one kilometre.

The NMDS ordination (Figure 3.6) revealed a similar result to the cluster analysis. *Zieria* sp. aff. *smithii* and *Z. smithii* overlap in ordination space. *Zieria prostrata* forms a distinct group, apart from the *Z. smithii* individuals which share a genotype with *Z. prostrata.*

A NMDS ordination on the combined morphological and genetic data set (Figure 3.7) revealed obvious clustering of taxa. *Zieria prostrata* clearly forms a distinct and discrete group, separated by a gap in phenetic space from the remaining taxa. *Zieria* sp. aff. *smithii* and *Z. smithii* are largely distinct but cluster adjacent to each other.

The combined data set also reveals a fourth cluster, comprising the three Beacon Hill *Z. smithii* individuals (top left of Figure 3.7). These Beacon Hill individuals are even more distant within the ordination space from *Z. smithii* as *Z. prostrata* is from *Z.* sp. aff. *smithii.* These Beacon Hill individuals were distinct but not discrete based upon the

morphometric data alone. Based upon the genetic data set, they came out highly distinct and discrete, due mainly to the presence of three population specific markers.

Figure 3.5 Dendogram from UPGMA cluster analysis of genetic data for *Zieria prostrata* (□), *Z. smithii* (●) and Z. sp. aff. *smithii* (○).

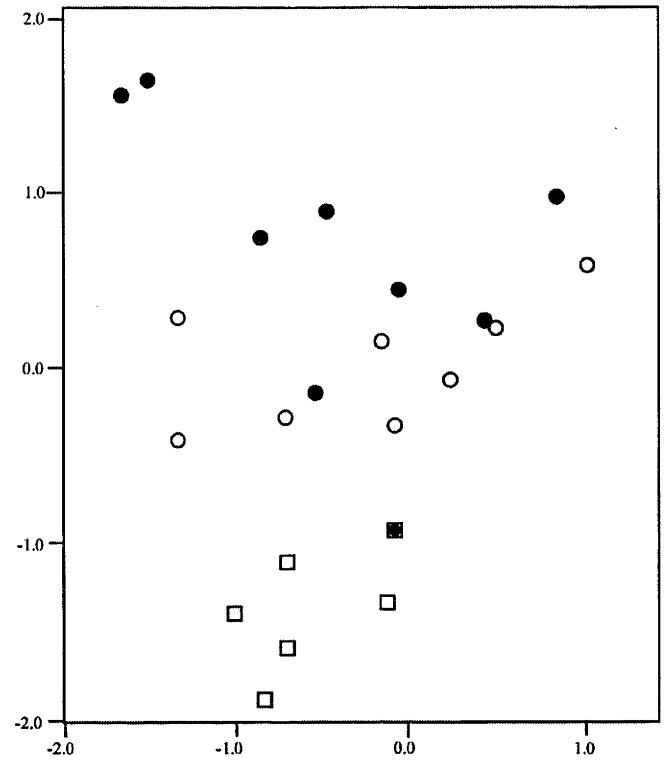

Figure 3.6 Two dimensional ordination (NMDS) of genetic data for *Zieria prostrata* (□), *Z. smithii* (●) and *Z.* sp. aff. *smithii* (○). Stress = 0.129.

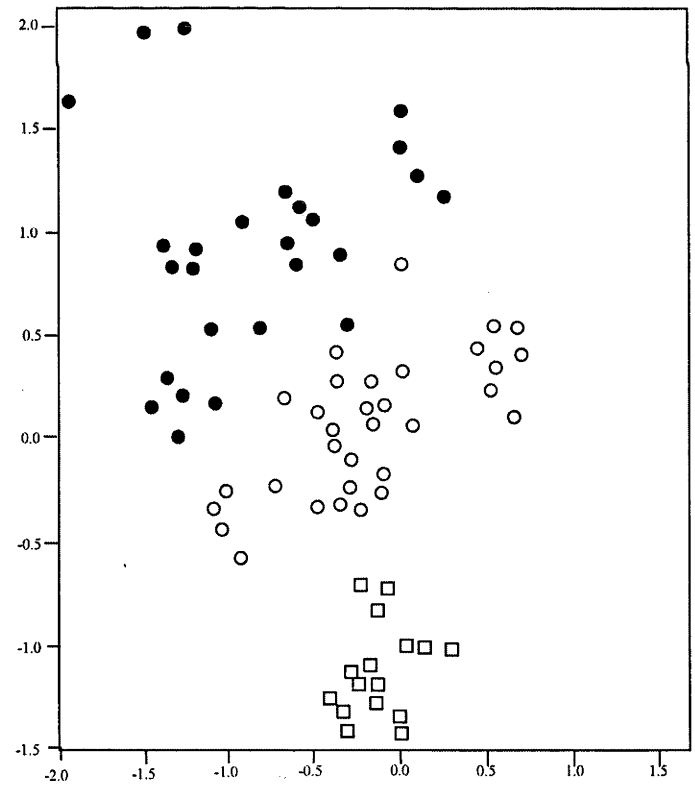

Figure 3.7 Two dimensional ordination (NMDS) of the combined morphometric and genetic data combined for *Zieria prostrata* (□), *Z. smithii* (●) and *Z.* sp. aff. *smithii* (○). Stress = 0.112.

3.4 Discussion

3.4.1 Morphological and genetic variation among study taxa

Zieria prostrata was found to be morphologically distinct from *Z.* sp. aff. *smithii* and *Z. smithii,* with *Z. prostrata* forming a distinct and discrete group in both ordination and cluster analysis. The distinction between *Z.* sp. aff. *smithii* and *Z. smithii* was less apparent. The two taxa exhibited obvious morphological differentiation, however there was some overlap between the two taxa in phenetic space.

The preliminary genetic research revealed that the relationship among *Z. prostrata*, *Z. smithii* and *Z.* sp. aff. *smithii* is more complex than indicated by the morphometric evidence alone. *Zieria prostrata* formed a single group in both ordination and cluster analysis space, as in the morphometric analysis, however, individuals from the nearby inland populations of *Z. smithii* also occurred within the group. The presence of five *Z. smithii* individuals within the *Z. prostrata* group is attributable to their genotype being indistinguishable from that of some *Z. prostrata* individuals based upon the small set of genetic markers. This overlap would most likely be removed with the inclusion of additional markers, given that detailed RAPD analysis on *Z. prostrata* alone revealed high population differentiation (Section 2.4.1). This trend of headland populations being genetically similar to nearby *Z. smithii* populations also appeared within *Z.* sp. aff. *smithii* and contributed to a lack of genetic differentiation between *Z. smithii* and *Z.* sp. aff. *smithii*, in contrast to the obvious morphological differentiation.

Such contrast between analyses of DNA and morphology in the estimation of taxonomic relationships is not uncommon (Dolan 1995; Hillis 1987; Hopper 1993; Sytsma 1990). Morphological evidence may fail to accurately represent evolutionary history due to the occurrence of phenotypic plasticity, introgression, convergence or parallelism, and active or rapid speciation (Section 3.1.3.3). In this case, it is unlikely that phenotypic plasticity, whereby morphological traits vary solely as a function of the environment (Donoghue and Sanderson 1992), is responsible for the discrepancy. For example, the Bongil Bongil National Park population of *Z.* sp. aff. *smithii* now occurs within typical *Z. smithii* habitat on an escarpment that is approximately four kilometres from the ocean, but was likely to be a headland less than 1.8 million years ago (Section 3.2.1). This population has maintained the phenotype of the headland *Z.* sp. aff. *smithii* populations, implying that the morphology of *Z.* sp. aff. *smithii* is not environmentally influenced or plastic. Additionally, *Z.* sp. aff. *smithii* plants grown from seed retain the typical *Z.* sp. aff. *smithii* form when grown in cultivation (pers. obs.). As outlined in the subsequent discussion on the evolutionary history of these populations (Section 3.4.2), the contrast detected in this study between the morphological and genetic evidence is

likely to be due to either introgression or, more likely, a combination of parallelism and active or rapid speciation. The discrepancy between the morphological and genetic evidence highlight the importance of using more than one class of character when attempting to infer phylogenetic relationships.

Irrespective of the contrast between the genetic and morphometric data set in terms of the relationship between *Z.* sp. aff. *smithii* and *Z. smithii*, the combined data set (Figure 3.7) provided a strong picture of the relationship among all three taxa. *Zieria prostrata* clearly forms a distinct and discrete cluster, separated by a gap in phenetic space from the remaining taxa. *Zieria* sp. aff. *smithii* and *Z. smithii* are largely distinct but cluster adjacent to each other. This distinction between *Z.* sp. aff. *smithii* and *Z. smithii* may not have been so strong if all the Bellangry State Forest and three of the five Alum Mountain individuals had not been excluded from the genetic analysis due to the presence of PCR inhibitors (Section 3.2.3.1), as these two populations were the predominant cause of overlap between *Z.* sp. aff. *smithii* and *Z. smithii* in the morphometric analyses. The combined data set also revealed a fourth distinct and discrete cluster, comprising the three Beacon Hill *Z. smithii* individuals.

3.4.2 Evolutionary history of these taxa

The genetic similarity between headland populations of both *Z. prostrata* and *Z.* sp. aff. *smithii* and nearby inland *Z. smithii* populations may be explained by two alternate hypotheses: (i) introgression, or gene flow between the headland and inland populations, or (ii) the headland populations are derived from the inland populations. Considering the first of these two hypotheses, if introgression is the cause of genetic similarities, this would mean the headland populations originated from a single population, spread along the coast, then introgressed with adjacent inland populations of *Z. smithii*. This scenario cannot be ruled out, however the most parsimonious explanation is that that headland populations repeatedly and independently originated from nearby inland *Z. smithii* populations.

The morphological distinctiveness of these headland populations, despite genetic similarities with nearby inland populations, implies that active or rapid speciation is taking place, whereby morphological differentiation is occurring at a greater rate than genetic differentiation (Schaal *et al.* 1991). This implies strong selection pressures for morphological changes that increase the survival of plants in the more hostile coastal environment. A similar contrast between the rate of morphological and genetic evolution has been shown in the Hawaiian Bidens (Asteraceae) as a result of radiating evolution (Ganders and Nagata 1984). Based upon morphology, taxonomists recognise nineteen species and eight subspecies of Hawaiian Bidens. However, the extensive

morphological diversity is not matched by genetic diversity, with each of the Bidens species being as genetically similar as populations of most other species.

So why then, are each of these headland populations of *Z.* sp. aff. *smithii* morphologically similar when the most logical idea is that each was independently derived from the morphologically distinct putative parent *Z. smithii*? These similarities are likely to have arisen through the evolutionary process termed parallelism, the development of similar features separately in two or more genetically similar, fairly closely related lineages (Sivarajan and Robson 1991). The occurrence of parallelism can be influenced by both the genotype of the ancestral taxon and the environment. Such closely related groups (i.e. all derived from *Z. smithii*) can evolve in similar patterns because they have similar evolutionary potentialities and are likely to produce similar mutations (Sivarajan and Robson 1991). Given the similar habitat and environmental conditions imposed upon each headland, each headland population is likely to have been subject to similar environmental, and thus selection pressures. In response to the similar selection pressures, natural selection has directed the headland populations towards a similar endpoint. This similar endpoint arose upon at least ten separate headlands and included a shift from the parental *Z. smithii* to a decrease in height, shorter and broader leaves and a reduction in hair density. That genetic differentiation between the inland and headland populations was not as great as the morphological differentiation implies that these adaptations are determined by relatively few genes (Gottlieb 1984).

The morphological similarity between the headland *Z.* sp. aff. *smithii* and the mountain top populations of *Z. smithii* (Alum Mountain and Bellangry State Forest) may also be a result of parallelism. These two mountain top populations of *Z. smithii* occur within exposed habitats, not typical of that supporting the 'true' *Z. smithii* and could thus be evolving independently and under different selection pressures. The morphological similarities between these mountain top populations and the headland *Z.* sp. aff. *smithii* populations may be a reflection, not only of similar evolutionary potentialities, but also similar selection pressures. Perhaps these exposed mountain tops receive harsh conditions, similar to those imposed upon headlands.

3.4.3 Taxonomic conclusions

As outlined in Section 3.1.3.2, within this study, a particular entity is recognised as a species when it forms a distinct and discrete unit in phenetic space, and as a subspecies when it forms a distinct, but not discrete unit. Final taxonomic conclusions are based upon the combined morphometric and genetic data set (Figure 3.7), keeping in mind the

patterns revealed by the genetic data (Figure 3.5), to ensure that recognised species represent distinct evolutionary lineages.

Overall, *Z. prostrata* formed a distinct and discrete unit in phenetic space, with a complete discontinuity between it and the remaining taxa. Therefore, *Z. prostrata* is indeed a distinct species. *Zieria prostrata* can be distinguished by its prostrate habit, shorter central leaf length, shorter petiole length, lower hair density on both leaves and stems, shorter inter leaf length, lower density of stem warts, and the presence of stem ridges. Speciation of *Z. prostrata* perhaps occurred relatively recently, explaining the genetic similarity between *Z. prostrata* and nearby populations of *Z. smithii.*

In the analysis of the combined morphometric and genetic data set, Z. sp. aff. *smithii* and *Z. smithii* formed distinct, but not discrete units in phenetic space. Following the operational definition, such taxa deserve subspecific rank. However, the analysis of the genetic data revealed considerable overlap between the two taxa. Therefore, given that *Z.* sp. aff. *smithii* does not represent a single lineage, the taxon is merely a headland ecotype of *Z. smithii.* It is likely that each of the headland populations evolved independently from inland *Z. smithii* populations, much as did *Z. prostrata.* However, in contrast to *Z. prostrata*, these populations have not yet diverged sufficiently to be considered distinct species. So for now they are convergent with *Z. smithii*, but given sufficient time, isolation and selection, any of these populations may diverge further from *Z. smithii* and evolve into distinct species. While not sufficiently distinct to justify specific status, each of these headland populations are highly important in their own right and are most likely in the process of speciation. The importance of these headlands populations should be recognised through a descriptive informal name, and I have proposed '*Zieria smithii* headland ecotype'.

Unexpectedly, this study also revealed a probable additional taxonomic entity. The three Beacon Hill *Z. smithii* individuals sampled formed a distinct and discrete unit in phenetic space in the analysis of the combined data set, even more discrete than *Z. prostrata.* This distinctiveness can be attributed to both genetic and morphological distinctiveness, but was greatly enhanced by the genetic data due to the detection of population specific markers. Additional sampling of this population and other nearby *Z. smithii* populations would be necessary in order to make any conclusions about the taxonomic status of this population.

3.4.4 Conservation implications

3.4.4.1 *Zieria prostrata*

This research has provided three important conservation implications for *Zieria prostrata.* Firstly, the status of *Z. prostrata* as a distinct species has now been confirmed. Secondly, the restricted distribution of *Z. prostrata* (only four sites across three kilometres) has also been confirmed, thereby confirming the species is worthy of its endangered status. Finally, this study revealed that *Z. prostrata* is likely to be an incipient species (i.e. of recent in origin). Provided adequate potential habitat is available, individuals of an incipient species may become abundant, alternatively, an incipient species may never become very abundant and may always remain rare, and therefore may continually be threatened by extinction (Watson *et al.* 1994). It is likely that *Z. prostrata* has always been rare, and will remain so, given the available habitat area on the four headlands and that the restricted gene flow between the headlands (Section 2.4.1) implies colonisation of other headlands is very unlikely to occur. Given that *Z. prostrata* is likely to have always been rare, range expansion via translocation to other headlands may be considered inappropriate.

3.4.4.2 The *Zieria smithii* headland ecotype

The *Z. smithii* headland ecotype, previously referred to as *Z.* sp. *aff. smithii,* was found to be part of the morphologically diverse *Z. smithii* species complex. Nonetheless, given the apparent independent origin of each of these headland ecotype populations and that these populations are clearly morphologically distinct from *Z. smithii*, this ecotype is obviously very important and worthy of conservation. The *Z. smithii* headland ecotype populations are distinct from *Z. smithii*, just not sufficiently so to justify status as a distinct species. Additionally, as mentioned in Section 3.4.3, given sufficient time, isolation and selection, any of these populations may diverge further from *Z. smithii* and evolve into distinct species, as did *Z. prostrata.*

So how do we go about conserving these important headland populations? One option is to list some of the most threatened populations in the Schedules of the *Threatened Species Conservation Act 1995* as threatened populations of *Z. smithii.* This has already been done for the Diggers Head population of *Z.* sp. aff. *smithii.* Such listing could help highlight the importance of conserving these headland habitats. Realistically, given that it is highly unlikely that any populations of *Z.* sp. aff. *smithii* will be subject to development proposals, given the location and zoning of populations, such listing or formal recognition is unlikely to improve the management of the populations beyond that which can be currently achievable. Of the ten populations of *Z.* sp. aff. *smithii* included in this research, four are protected within National Parks. The remaining six

populations occur upon Crown Land, four of these populations occur upon land zoned *7 (F1) Coastal lands protection* and two occur upon land zoned *6 A-Public recreation.* One of these later two populations, Diggers Head, is already listed as a threatened population. Therefore, only one population, Valla Headland, is not currently formally protected in some form. Therefore, attempting to achieve formal recognition of Z. sp. aff. *smithii* is probably not a priority and efforts may be best focussed upon alleviating threats to the populations. The predominant threat to the majority of Z. sp. aff. *smithii* populations is displacement by Bitou Bush (*Chrysanthemoides monilifera*). Given that the majority of these headlands are already managed by local councils or the NSW National Parks and Wildlife Service, monitoring of the *Z. smithii* headland ecotype populations and management to control Bitou Bush and any other potential threats could be undertaken as part of the already existing management programs.

3.4.5 Conclusions

The main objective of this chapter was to clarify the taxonomic status of *Zieria prostrata. Zieria prostrata* was confirmed to be worthy of specific status. Based upon the combined morphometric and genetic data, *Z. prostrata* was found to be distinct from *Z.* sp. aff. *smithii* and *Z. smithii,* with *Z. prostrata* forming a distinct and discrete unit in phenetic space, with a complete discontinuity between it and the remaining taxa. The genetic evidence suggests speciation of *Z. prostrata* perhaps occurred relatively recently, with genetic similarities between *Z. prostrata* and nearby populations of *Z. smithii.*

This research has provided three important conservation implications for *Zieria prostrata.* Firstly, the status of *Z. prostrata* as a distinct species has now been confirmed. Secondly, the restricted distribution of *Z. prostrata* has also been confirmed, thereby confirming that the species is worthy of its endangered status. Finally, this study revealed that *Z. prostrata* is likely to be an incipient species and thus may have always been rare. Therefore range expansion via translocation to other headlands may be considered inappropriate.

CHAPTER 4

Reproductive ecology of *Zieria prostrata*: factors limiting recruitment and management options which may be used to overcome limiting factors

4.1 Introduction

4.1.1 Background

In the preceding chapter, it was confirmed that *Zieria prostrata* is a distinct species restricted to only four headlands. With abandonment of the proposed reintroduction of *Z. prostrata* to site 5 in 1996 (Section 2.4.2), the priority for management became the protection of the four extant populations. The genetic research outlined in Chapter 2 provided a number of management recommendations for the conservation of these populations based upon genetic concerns (Section 2.4.3). For example, these included the recommendation that population distinctiveness be retained during any population enhancement work. However, these recommendations primarily reflect concerns about the long-term persistence of populations. Demographic factors, on the other hand, are considered to be of more immediate concern for the survival of threatened species (Caro and Laurenson 1994; Lande 1988; Schemske *et al.* 1994; Widen 1993).

In 1995 all known populations of *Z. prostrata* were incorporated within an adjacent nature reserve (Section 1.2). Is such habitat reservation sufficient to ensure demographic stability and population persistence for this species? As a species of recent origin, *Z. prostrata* may have always had a highly localised distribution (Section 3.4.4.1). Given the area of the headlands, it is unlikely that the past abundance of *Z. prostrata* was ever considerably greater than its current abundance. Indeed, it is even feasible, given the species relatively recent origin, that the species has not yet reached its full potential and population size is still increasing. Irrespective of the exact past abundance of the species, these populations have persisted up until now, despite presumably facing both the demographic and environmental stochasticity associated with such a restricted distribution and small population sizes. The question is, will these populations continue to persist without human intervention? If not, how can we restore or maintain population vigour? The answer to both these questions lies in an understanding of the reproductive ecology of the species.

4.1.2 Reproductive ecology and conservation

4.1.2.1 Limiting factors and population stability

Knowledge of the reproductive ecology and critical life history stages of a threatened plant population can provide vital insight into population stability. The level of recruitment is the primary determinant of population stability, given that a failure to produce enough new recruits to replace adult deaths will decrease population stability and eventually lead to population extinction. The stability of populations of threatened plants can thus be jeopardised by limits to reproduction.

There are numerous phases in a plant's life cycle where limits to reproduction may occur. For example, in a plant that relies solely on sexual reproduction, flowers must be pollinated, ovules must be fertilised and sustained with nutrients, and fruits must escape predation until viable seeds are formed. These seeds then need to be dispersed to suitable substrates for growth, where they must germinate and the resultant seedlings need to survive and reproduce. Any weak link or break in this chain of events reduces a plants ability to reproduce and, if constant over space and time, may threaten population persistence (Kaye 1999). Not only are there numerous phases within a life cycle that may be subject to limits, but there are also a diverse range of factors which may impose these limits. For example, in the single stage of seed production, the output of viable seed may be limited by a diverse array of factors including: pollinator limitation (Charlesworth 1989; Groom 1998; Jennersten 1988; Menges 1991; Pavlik *et al.* 1993), predation (Louda 1982a, 1982b, 1983, 1989; Louda and Potvin 1995), resource limitation (Campbell and Halama 1993; McCall and Primack 1985; van Andel and Vera 1977), and self-incompatibility when combined with small population size or extensive clonality (Demauro 1994; Negron-Ortiz 1998; Thien *et al.* 1983).

It needs to be noted, however, that evidence of limits to a particular life cycle stage does not mean that this limit is reducing population recruitment or that limits to population recruitment are limiting population stability. Some factor operating during the life cycle must regulate the number of individuals within a population. Not all flowers produce fruit, not all fruit produce seed, not all seed germinate, and so on. Only when a limit reduces recruitment beyond that required to replace adult deaths is population stability jeopardised. Additionally, the impact of limits to a stage of a plants life cycle may be negated by limits to a subsequent stage. For example, Andersen (1987) found that predation of *Eucalyptus baxteri* and *Casuarina pusilla* seeds by ants was decreasing the number of viable seed considerably. Elimination of seed eating ants increased the number of seedlings 15-fold in *Eucalyptus baxteri*, and four-fold in *Casuarina pusilla.* However, all seedlings ultimately perished, and consequently the seed losses to ants had

no apparent impact upon recruitment since seedling survival, not predation, was the limiting factor. In another example, Calvo (1993) showed that in *Tolumnia variegata,* fruit production is severely pollinator limited. However, as recruitment is limited by safe site availability, i.e. the availability of sites suitable for seedling recruitment and survival, pollinator limitation alone does not restrict the species.

4.1.2.2 Overcoming limiting factors: identifying management options

Understanding the reproductive ecology of a threatened plant can also help identify management options which may be used to overcome limits to reproduction and to thus potentially stabilise or increase population size (e.g. Hegazy 1990; Lesica 1992; Morgan 1995; Pavlik *et al.* 1993; Pavlik and Manning 1993). If thorough investigation of the reproductive ecology of a species reveals no major limits to reproduction, it may be inferred that the population(s) are relatively stable, provided they are large enough to avoid both demographic and environmental stochasticity. In such instances habitat protection may be sufficient to ensure the survival of the species. Alternatively, if major limits to recruitment are identified, then manipulative management may be used to overcome or minimise the limits imposed upon the critical life history stage(s). Manipulation of the factors that limit rare species is recognised as a useful, relatively low cost tool for the management and restoration of threatened plant species (Bevill *et al.* 1999; Keddy *et al.* 1989).

Knowledge of reproductive limits has been used to guide management actions for a range of plant species. For example, Pavlik *et al.* (1993) found pollinator limitation reduced seed set in the endangered plant *Qenothera deltoides* ssp. *howellii* and recommended management efforts protect the local populations of pollinators in order to minimise pollinator limitation in the future. In another example, seed production in the threatened self-incompatible plant *Aster furcatus* was found to be restricted by the limited number of mating types, or S alleles, available within the small populations (Les *et al.* 1991). The introduction of new individuals, containing distinct S alleles, was recommended as one strategy to increase seed production.

There are also examples of where the identification and removal of limits to reproduction has successfully led to an increase in recruitment and population size. For example, Menges (1995) found that seedling emergence of the forb *Silene regia* increased two- to three-fold after soil disturbance or burning. Prescribed burning at a number of sites not only led to an increase in *Silene regia* population sizes, but also restored ecosystem structure and function. In another example, Bevill *et al.* (1999) found that experimentally excluding insect herbivores from juvenile rosettes of the

threatened Pitcher's thistle (*Cirsium pitcheri*) led to a decrease in juvenile mortality and an increase in seed production for those juveniles that matured and flowered.

Nonetheless, given that evidence of limits to a stage in the life cycle of a plant does not guarantee the limits are reducing population recruitment (Section 4.1.2.1), the removal of a limit to reproduction will not always lead to an increase in recruitment and population size. For example, in a previously mentioned study, Calvo (1993) showed that fruit production in *Tolumnia variegata* was severely pollinator limited, but recruitment was limited by safe site availability (Section 4.1.2.1). Therefore, increasing fruit set in this species via manipulative management, such as increasing pollinator density or hand pollination, may not lead to an increase in recruitment, only an increase in safe site availability could potentially increase population recruitment. This complication demonstrates the importance of investigating, or at least understanding, all the sequential stages in the life cycle of a plant and the interactions between each stage, if the critical factor limiting recruitment is to be identified.

4.1.3 Identifying stages in the life cycle of *Zieria prostrata* requiring detailed research

Figure 4.1 depicts those stages in the life cycle of *Zieria prostrata* where limits to reproduction may be imposed. For example, flowers may not be fertilised due to pollinator limitation, seeds may fail to develop due to pre-dispersal seed predation or fruit abortion, dispersed seeds may be lost to predators or they may not reach a safe site, seeds may fail to germinate due to an absence of safe sites, or seedlings may not persist. A detailed investigation of all these stages was not possible in the time frame imposed upon this research. Therefore, preliminary investigations were conducted in order to identify those stages that were likely to be the most important. Further research therefore targeted those stages where reproductive limits were most likely.

4.1.3.1 Outcomes of preliminary investigations

Zieria prostrata appears to be a long-lived perennial with a relatively stable adult population. A number of the plants established during the 1993 *ex-situ* program (Section 1.2) were still healthy, flowering and producing fruit eight years after establishment. Comparison of the basal stem diameter of these *ex-situ* plants with those of plants within the natural populations suggests that many plants in the wild are likely to be at least three times older, implying plants may live for up to at least 20 years. Further evidence supporting the stability of the adult population was obtained through a demographic monitoring study, established in 1996, which followed the fate of 63 randomly selected adult *Zieria prostrata* plants from across all four populations (20 from each of sites 1, 3 and 4 and three from site 2). Only one death was observed over

two and a half years of monitoring. Unfortunately, plant survival could not be monitored over a longer time period as the plant and transect markers were disturbed by vandals, preventing accurate relocation of plants.

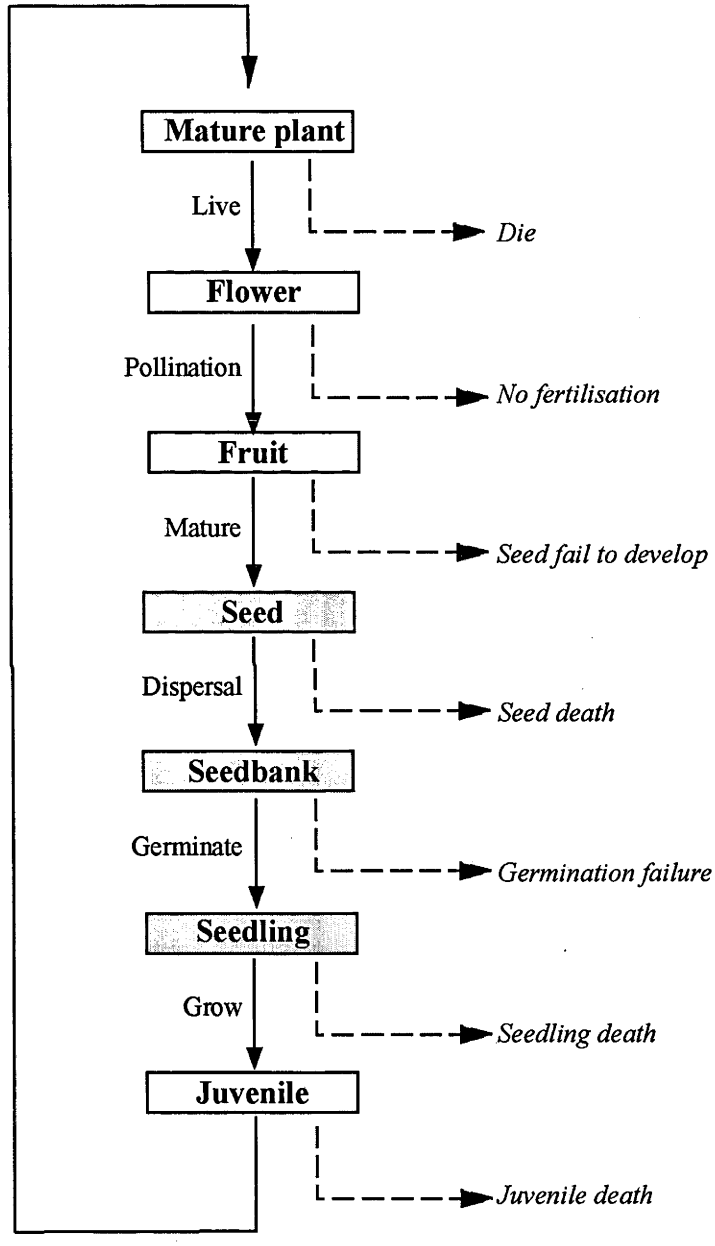

Figure 4.1 A diagrammatic representation of the life cycle of *Zieria prostrata* illustrating the various stages where limits to reproduction may occur. Each rectangle represents a pool of individuals at each life cycle stage. The solid line indicates successful transition from one stage to the next, whereas the broken lines represent loss's which may occur due to the factors in italics. Those stages examined in detail in this chapter are shaded.

There are also no apparent limitations to flower production or pollen viability in *Zieria prostrata*. Each year *Z. prostrata* produces abundant flowers, with plants covered in masses of flowers from late August until early October. All the 63 adult *Z. prostrata* plants monitored flowered over two consecutive seasons. Despite the speculation of Armstrong (ms) that *Z. prostrata* is male sterile, a preliminary investigation of pollen viability, using the flurochromatic reaction (FCR) test (Heslop-Harrison and Heslop-Harrison 1970), following the methods outlined by Kearns and Inouye (1993), found no evidence of pollen sterility. Five individuals from each of the four populations were assessed, with mean (± SE) pollen viability ranging from 82 ± 0.9% to 96 ± 0.5% across the populations.

There also appears to be no limit to seed set in *Zieria prostrata*. Despite a lack of obvious insect pollination in the field, plants are covered in masses of fruit from late September until early December. This suggests the species may be capable of self-pollination in the absence of pollinator activity (autogamy). Indeed, further research confirmed that *Z. prostrata* is self-compatible and capable of autogamy. A glasshouse pollinator exclusion and hand pollination trial revealed that seed set occurred in 30% (n = 50) of flowers excluded from pollinator activity. Artificial pollination yielded 96% (n = 32) seed set for selfed flowers and 76% (n = 101) for cross-pollinated flowers.

The ability of *Z. prostrata* to self-pollinate in the absence of pollinator activity can be attributable to flower morphology, with the anthers dehiscing directly above the stigma. It appears autogamy is common within the *Zieria* genus, as Armstrong (ms) found 64% of *Zieria* species examined, including the closely related *Z. smithii*, produced fruit when their flowers were isolated from pollinators. Additionally, the distribution of genetic variability among populations of *Z. prostrata* suggests that there is a high level of self-pollination within the natural populations (Section 2.4.1).

4.1.3.2 Factors requiring detailed research

The preliminary investigations reported above indicated that there were no major limits to mature plant survival, flowering, fruit set and seed production in *Zieria prostrata*. However, limits to reproduction in those subsequent stages from seed viability through to juvenile survival required detailed investigation. Previous evidence suggested that seed predation might limit the output of viable seed in this species. Prakash (1995) found nearly 50% of *Z. prostrata* seeds examined had been consumed by an unknown insect larvae. Furthermore, extensive survey within the first year of this study located only three seedlings, all arising from a small disturbed area on a cliff face. Therefore the life cycle stages from seed viability through to seedling survival form the focus of this chapter (Figure 4.1).

4.1.4 Chapter objectives

The primary objective of this study was to investigate the reproductive ecology of *Zieria prostrata*, focusing upon seed viability through to seedling survival, in order to: (i) determine if there are any factors limiting recruitment, and (ii) identify management options capable of increasing recruitment.

More specifically, five distinct aspects of the reproductive ecology of *Zieria prostrata* were investigated and the following questions were addressed:

1. Seed viability: Does *Z. prostrata* produce viable seed? Does seed predation have a significant impact upon the output of viable seed?

2. Seed germinability: Are *Z. prostrata* seeds capable of germinating? Do *Z. prostrata* seeds possess a dormancy mechanism? Does the level of light or smoke play a role in breaking seed dormancy?

3. Seed bank dynamics: Does *Z. prostrata* maintain a soil stored seed bank? If so, how persistent is it? Does seed burial or canopy cover play a role in seed bank longevity and seed germination?

4. Disturbance and germination: Does disturbance increase seedling recruitment? Does the timing of disturbance or the distance of disturbance from an adult plant influence recruitment?

5. Seedling survival: Are *Z. prostrata* seedlings capable of persisting?

4.2 Methods

4.2.1 Seed viability and predation levels at release

To investigate the viability of *Zieria prostrata* seed, seeds were collected from 20 randomly selected individuals at sites 1, 3 and 4, and from three individuals at site 2, in both 1996 and 1997. The seed of *Z. prostrata* are released balistically upon fruit maturation in late November to early December. When fruits were nearly mature, in late October of each year, nylon mesh bags were tied onto one randomly selected fruiting branch of each plant, enabling capture of dispersed seed. The bags and their enclosed seed were retrieved in late December. Seeds were not obtained from three plants at site 3 and from four at site 4 in 1996 due to the removal of seed bags by vandals prior to collection. Thirty seeds per plant were scored for viability and predation. Eaten seeds were obvious due to the presence of a larval exit hole and the viability of intact seeds was scored by dissection and visual inspection. Those seeds containing white fleshy embryos were scored as viable and those containing shrunken and/or or discoloured embryos were scored as inviable.

The following, and all subsequent analyses within this chapter were performed using the analysis package JMP® (SAS Institute Inc. 1994). Variation in seed viability both among sites (n = 4, fixed) and among years (n = 2, fixed) was investigated using a two-factor analysis of variance (ANOVA). The proportion of viable seed per plant was arcsin transformed and homogeneity of variances was tested using the Bartlett's test. Given the extremely low variances within sites 1 and 2 (due to 97-100% seed viability), variances were not homogeneous among populations. Heterogeneous variances can increase the probability of a Type 1 error (rejecting the null hypothesis when it should be accepted) (Underwood 1997). However, I chose, after evaluation of the outcome, to accept the results of the ANOVA despite heterogeneous variances, given that the results were either non-significant or highly significant. When a non-significant difference among factors is obtained, then the result is valid because a Type 1 error cannot be made (Underwood 1997). When a significant difference is detected, the chances of performing a Type 1 error can be minimised or removed by choosing a conservative probability value, for example of 0.01 (Underwood 1997). In this instance, even if an extreme significance value of 0.0001 was chosen to minimise the possibility of a Type 1 error, the outcome would not change. Post-hoc comparisons of site means were made using the Tukey-Kramer HSD (honestly significantly difference) test.

Variation in the level of seed predation among sites (n = 2, fixed) and years (n = 2, fixed) was also tested using a two-factor ANOVA. Only sites 3 and 4 were included in this analysis given the absence of predation at sites 1 and 2. The proportion of eaten

seed per plant was arcsin transformed and homogeneity of variances was confirmed using the Bartlett's test.

4.2.2 Seed dormancy and germination

A laboratory based seed germination trial was used to investigate the dormancy and germination characteristics of *Zieria prostrata* seed. Four month old seed, collected from all four populations, was pooled and the eaten seeds were removed. Initial seed viability was assessed for 80 seeds as outlined in Section 4.2.1 and was found to be 78%. Four treatments were included in the trial: (i) pre-treated with smoke water (diluted 1:10) (produced by Kings Park and Botanic Gardens, WA) and maintained in total darkness, (ii) no pre-treatment and maintained in total darkness, (iii) pre-treated with smoke water and maintained in a 12 hour light/dark cycle, and (iv) no pre-treatment and maintained in a 12 hr dark/light cycle. Each treatment comprised four replicates of 40 seeds, placed in 9 cm diameter petri dishes on Whatman Grade 2 filter paper moistened with distilled water. The total darkness replicates were wrapped in aluminium foil to exclude light. All replicates were placed in a growth cabinet maintained at a constant 20°C and set to a 12 hour light/dark cycle. The seeds were watered with distilled water when required. One month into the experiment, 1 ml of 2% Fongarid® (Chemspray Pty Ltd, Smithfield, NSW) was added to each petri dish to control minor fungal outbreaks. Germinants were recorded and removed at approximately five day intervals. The experiment was maintained for a total of 110 days. At the end of the trial, all ungerminated seeds were assessed for viability as outlined in Section 4. 2.1.

The proportion of seed germinated at 110 days for each replicate was calculated both as a proportion of the total number of seeds and as a proportion of the total number of viable seeds (i.e. inviable seeds excluded). For each data set, the proportion of seed germinated was arcsin transformed, homogeneity of variances was confirmed using the Bartlett's test, and a two-factor ANOVA was used to investigate the impact of light treatment (n = 2, fixed) and smoke water pre-treatment (n = 2, fixed) on seed germination. Differences in seed germination among the four treatments was further investigated using the post-hoc Tukey-kramer HSD test.

4.2.3 Seed bank dynamics

4.2.3.1 Natural seed bank

The size, viability and longevity of the soil stored seed bank of *Zieria prostrata* was investigated by collecting soil samples from under the canopy edge of five adult plants at site 3 at 0, 3 and 6 month intervals after seed fall. Soil samples, 300 cm^3 (10 cm x 10 cm surface area and 3 cm deep), were randomly sampled from each plant at each time

interval from one of four previously assigned positions under the plant canopy (N, S, E and W). Each soil sample was air dried for approximately five days. Samples were initially passed through a 2 mm sieve to remove the coarse material. *Zieria prostrata* seeds were then collected from the remaining soil by visual sorting. The collected seeds were scored as either viable, inviable or eaten as described in Section 4.2.1. The number of viable seed per sample for each time interval was square root transformed and homogeneity of variances was confirmed using the Bartlett's test. Variation in the level of seed viability among time intervals (n=3, fixed) was tested using an analysis of variance (ANOVA). The factor, plant (n= 5, random), was included to account for any seed bank density variation among the five plants.

4.2.3.2 Seed burial experiment

Given the apparent high spatial variability in seed bank density and the desire to avoid excessive sampling of the natural seed bank, the seed bank dynamics of *Z. prostrata* were investigated in greater detail using a seed burial and recovery experiment. This experiment was established in December 1996, so that germination, dormancy and viability of the seed bank over time could be investigated. Additionally, the burial experiment was designed to allow investigation of the influence of canopy cover and seed burial depth on the maintenance of a soil stored seed bank. The experiment involved burying seeds within *Z. prostrata* habitat (site 3) for varying lengths of time, at two depths (2 cm and 5 cm), either under the canopy of an adult plant or in a canopy gap.

Zieria prostrata seeds collected from various plants from all populations in 1996 were pooled and the eaten seeds were removed. Initial seed viability was assessed for four replicates of 25 seed and was found to be 76±2% (mean ± SE). Seeds were sewn into eighty, 3 cm x 3 cm, nylon mesh bags, with each bag containing 25 seeds. Four experimental plots were set up at site 3. Within each plot, ten bags were buried under the canopy of an adult plant and ten were buried in an artificially created canopy gap. The gaps were created by cutting and removing all existing vegetation to expose bare soil. Five of the ten bags were buried to a depth of 2 cm and five to a depth of 5 cm. Therefore each of the four plots contained four treatments: (i) disturbed: canopy removed and buried at a depth of only 2 cm (D-2cm), (ii) disturbed: canopy removed and buried at a depth of 5 cm (D-5cm), (iii) under the canopy of an adult plant and buried at 2 cm (C-2cm), and (iv) under the canopy of an adult plant at 5 cm (C-5cm). To enable collection of the bags without disturbing adjacent bags, the position of each bag was marked using a small wooden stake.

One randomly selected bag from each of the four treatments was collected from each of the four plots at approximately two month intervals over an 11 month period. Germinated seed were identified as either a seedling, or a split seed case when the seedling had perished. The remaining intact seeds were scored for viability as outlined in Section 4.2.1.

The proportion of seeds that had germinated and the proportion remaining dormant at 11 months, were arcsin transformed and homogeneity of variances for each data set was confirmed using the Bartlett's test. The influence of canopy cover (n = 2, fixed), burial depth (n = 2, fixed) and plot (n = 4, random), upon the number of both germinated and dormant seeds was investigated using a three factor ANOVA. Differences in seed germination and dormancy among each treatment were further investigated using the post-hoc Tukey-Kramer HSD test.

Climatic data for Coffs Harbour, approximately 14 kilometres south of the study site, including the maximum and minimum daily temperature and daily rainfall, was obtained for the entire study period (provided by the Australian Bureau of Meteorology) allowing comparison of the peak germination period with climatic conditions.

4.2.4 Impact of disturbance on germination

A disturbance experiment was used to further investigate the impact of disturbance on seedling recruitment. Six experimental plots were established within each of three populations (Sites 1, 3 and 4). Each plot was located at the edge of the canopy of an adult plant and was 90 cm x 40 cm and comprised 36 sub-plots, each 10 cm x 10 cm (Figure 4.2). Eighteen of these sub-plots were assigned a treatment and the remaining acted as buffer areas to minimise edge effects among treatments. Each plot was divided into three distance categories, 30 cm, 60 cm and 90 cm from an adult plant. Each distance category comprised six treatment sub-plots which were each randomly assigned one of six treatments; (i) disturbed time 1, (ii) control time 1, (iii) disturbed time 2, (iv) control time 2, (v) disturbed time 3, and (vi) control time 3. Therefore the effect of three factors upon seedling emergence; disturbance, timing of disturbance, and distance from adult plant, were investigated.

The experiment commenced in late November 1997, directly after seed release. The appropriate sub-plots were disturbed by cutting away all vegetation and turning the soil with a small hand spade. The small size of the disturbance area (10 cm x 10 cm) was used primarily for two reasons. Firstly, the creation of large disturbed areas was avoided due to concerns erosion may eventuate in larger areas. Secondly, such a small gap is likely to mimic those eventuating from natural disturbances. Gaps created by

disturbance within *Zieria prostrata* habitat are likely to be small-scale and caused by, for example; rock falls, water run-off after heavy rain, adult plant death or branch breakage, salt spray, and digging by small mammals. The second and third disturbance events were performed three and six months later in February and June of 1998 respectively. Each plot was monitored for seedling emergence at three monthly intervals for a minimum of six months (maximum of 12 months).

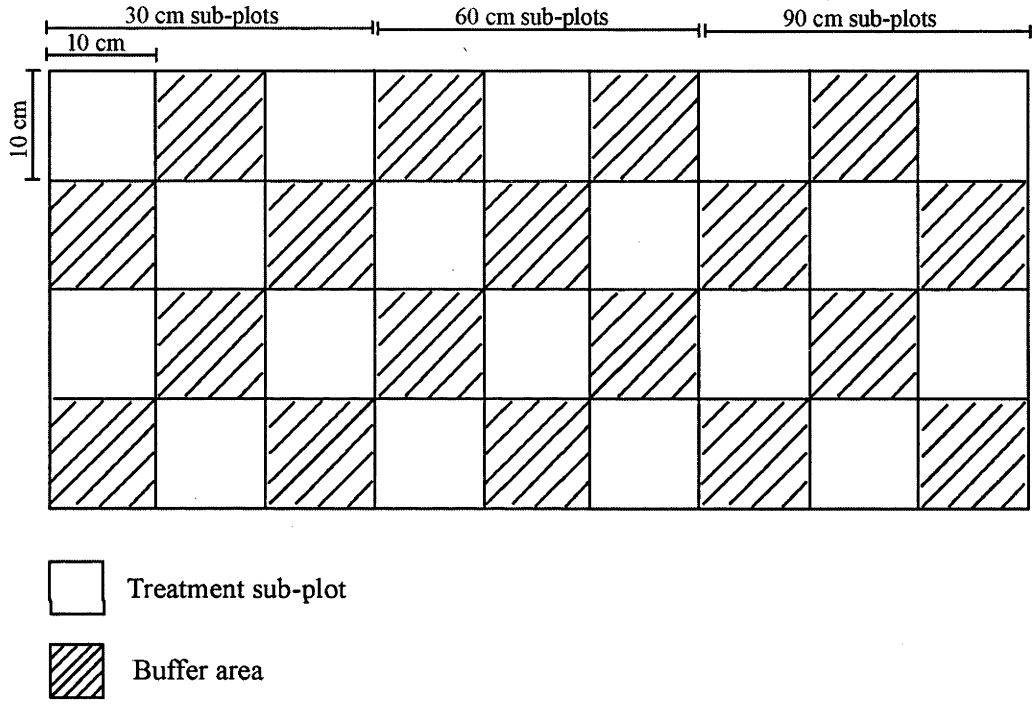

Figure 4.2 Schematic representation of the experimental plot design used in the disturbance experiment investigating the influence of disturbance, distance from adult plant and timing of disturbance upon seedling recruitment in *Zieria prostrata.*

Unfortunately, seedling emergence was apparent in only five of the 18 experimental plots over the entire study period. Analysis was therefore performed only on those five plots where germinants were recorded. An ANOVA was performed on the number of germinants present six months after treatment. The three factors; disturbance (fixed, n = 2), time of disturbance (fixed, n = 3), and distance from adult plant (fixed, n = 3) were included. Plot (n = 5, random) was also included as a block factor to test for any variance among plots in the number of germinants. Homogeneity of variances was tested using the Bartlett's test. Given the large number of zero germinants and the patchy distribution of germinants, variances were not homogeneous. Data transformation did not improve homogeneity of variances and thus analysis was

performed on the raw data. As outlined in Section 4.2.1, heterogeneity of variances can increase the probability of a Type 1 error (Underwood 1997). However, after evaluation of the outcomes, I chose to accept the results of the ANOVA despite heterogeneous variances, given that the results were mainly non-significant and when significant a conservative significance value of 0.01 was chosen to minimise the chances of a Type 1 error.

Given the unfortunately low level of seedling emergence, in January of 1999, after the 1998 seasons seed fall, a 300 cm^3 soil sample (10 cm x 10 cm surface area and 2 cm deep) was collected from two randomly selected buffer areas for each of the 18 experimental plots to investigate soil seed bank density, enabling a comparison with germination rates.

4.2.5 Seedling survival

Four plots (30 cm x 30 cm) were established in February 1998 to monitor seedling survival. Given that seedlings rarely occur naturally without disturbance, the plots were physically disturbed to promote seed germination. Within each plot, the canopy and all vegetation were removed and the soil was lightly turned. A total of 309 seedlings were recorded within all four plots three months after disturbance, in early June 1998. Each seedling was mapped and the plots were subsequently monitored four times over a total of 16 months. The percentage of original seedlings alive, within each plot, at each monitoring event, was plotted on a log scale to explore survivorship patterns.

4.3 Results

4.3.1 Seed viability and predation levels at seed release

A significant difference in seed viability was detected among *Zieria prostrata* sites ($F_{3, 104} = 102.914$, $P = 0.0001$), but not among years ($F_{1, 104} = 1.169$, $P = 0.282$) (Figure 4.3 a). No significant interaction between sites and years was detected. There was no significant difference in seed viability among sites 1 and 2, with all seeds from site 2 and the majority from site 1 (97-99%) being viable. In contrast, only 40-47% and 71-77% of seeds from sites 3 and 4 respectively were viable. This reduced level of viable seed for sites 3 and 4 was due to the presence of a high proportion of inviable and eaten seeds in both 1996 and 1997 at these sites (Figure 4.3 b and c). The level of seed predation varied among sites 3 and 4 ($F_{1, 64} = 41.059$, $P = 0.0001$) but not among years ($F_{1, 64} = 0.710$, $P = 0.403$). There was no significant interaction between sites and years. Seed predation also appeared to be plant specific. All site 3 plants possessed some eaten seeds in both 1996 and 1997. Whereas for site 4, no eaten seeds were recorded for eight plants in both years, despite being less than 100 meters from plants possessing eaten seeds.

4.3.2 Seed dormancy and germination

Zieria prostrata seeds were capable of germinating under all the conditions imposed with 47 ± 2.1% (mean ± SE) of seeds germinating across all treatments. Without correcting for the number of inviable seed, both darkness ($F_{1, 11} = 5.214$, $P = 0.043$) and smoke water pre-treatment ($F_{1, 11} = 9.590$, $P = 0.010$) appear to significantly increase seed germination (Figure 4.4). There was no significant interaction between pre-treatment and light regime ($F_{1, 11} = 3.804$, $P = 0.077$). Post-hoc comparisons revealed significantly more germinants only for those seeds pre-treated with smoke water and maintained in total darkness, and not for those seeds pre-treated and exposed to light, nor for those seeds not pre-treated and maintained in total darkness. Given this unexpected result, the data was re-analysed after excluding the large and variable number of inviable seeds (Figure 4.5). When the inviable seed are excluded, the promotive effect of smoke water and darkness is not apparent, with light treatment ($F_{1, 11} = 1.86$, $P = 0.202$) and smoke water pre-treatment ($F_{1, 11} = 2.191$, $P = 0.167$) having no significant effect upon seed germination. Again, there was no significant interaction between pre-treatment and light regime ($F_{1, 11} = 0.018$, $P = 0.896$). Not all viable seeds germinated, with 21 ± 2.63 % (mean ± SE) of seed remaining dormant after germination ceased (Figure 4.5).

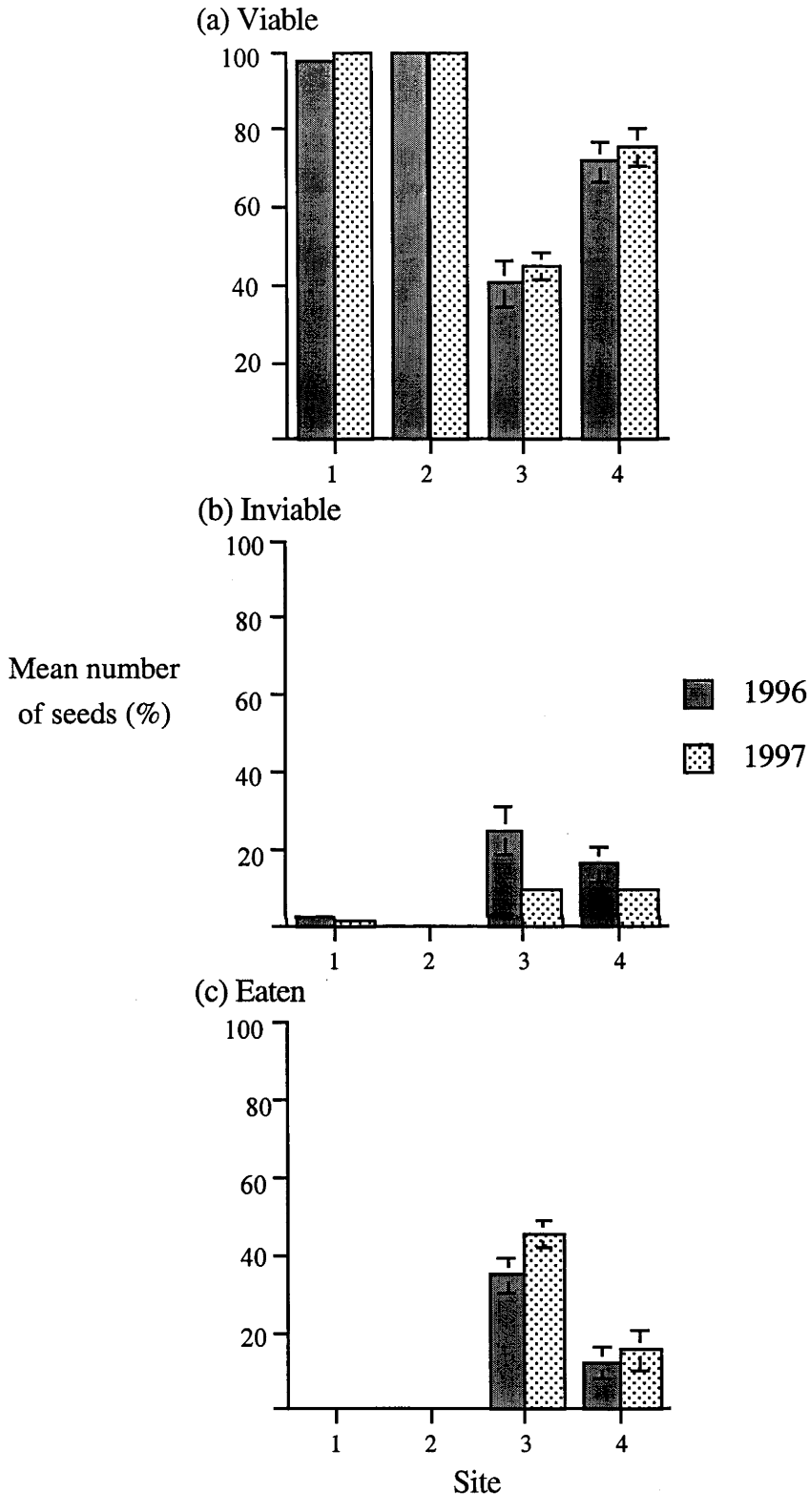

Figure 4.3 The mean percentage of (a) viable, (b) inviable, and (c) eaten seeds, collected at seed release from four populations of *Zieria prostrata* during 1996 and 1997. Error bars represent standard error.

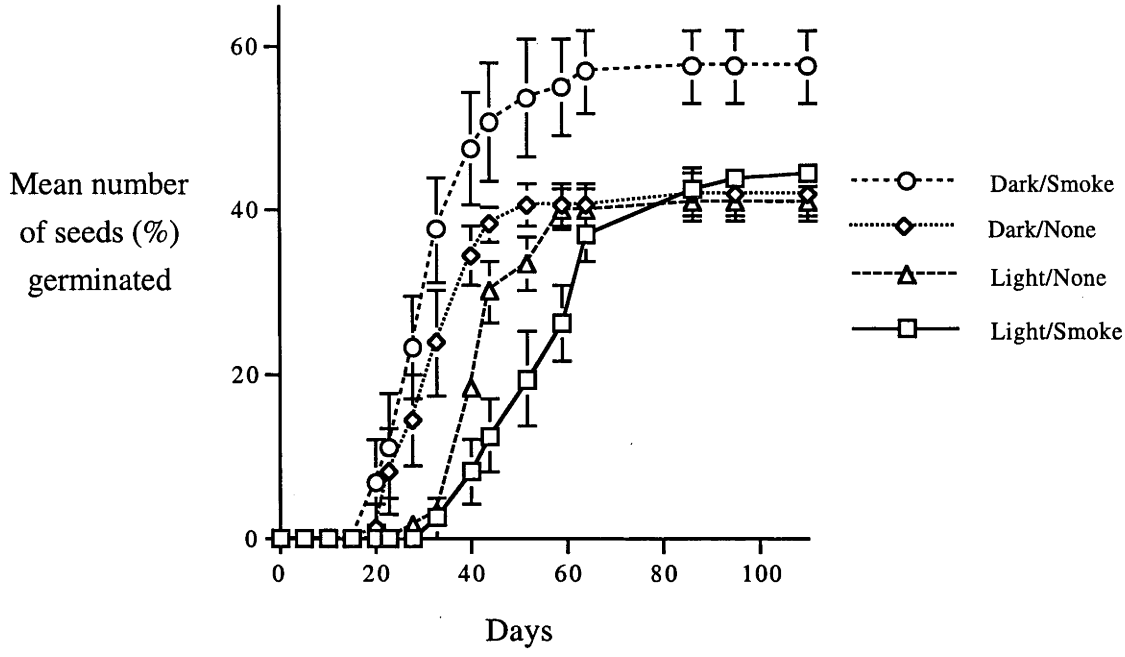

Figure 4.4 The mean germination rate for *Zieria prostrata* seeds subject to four different treatments. 'Dark' represents seeds maintained in total darkness, whereas 'Light' represents seeds maintained in a 12hr light/dark cycle. 'Smoke' represents seeds pre-treated with smoke water, whereas 'None' represents seeds not subject to any pre-treatment. Error bars represent standard error.

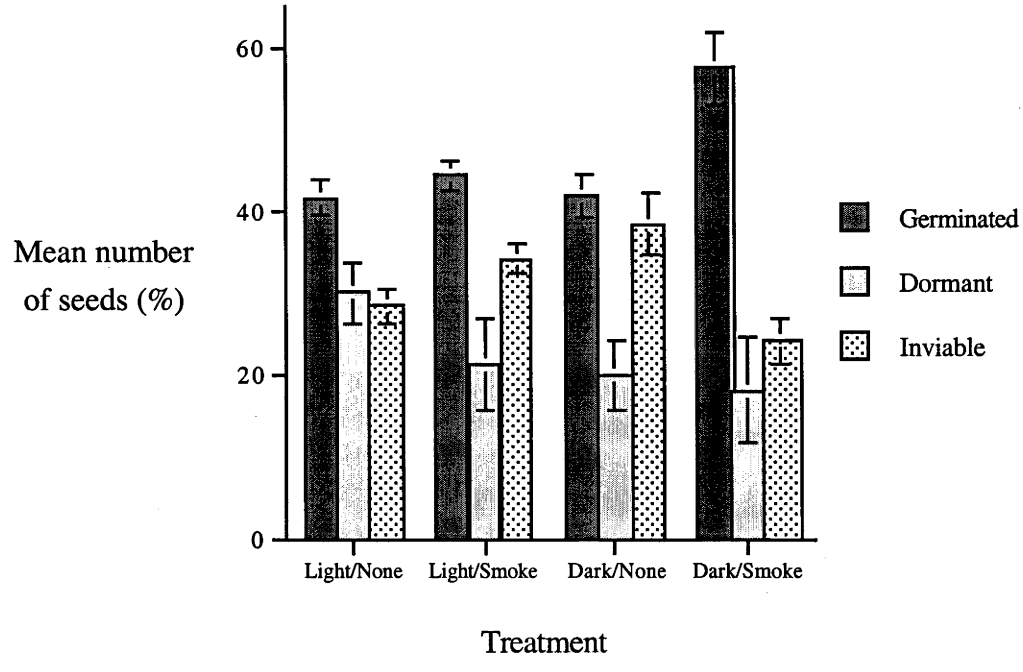

Figure 4.5 The mean percentage of *Zieria prostrata* seeds germinated, dormant and inviable after 110 days subject to four different treatments. Treatment abbreviation explanations as in Figure 4.4.

4.3.3 Seed bank dynamics

4.3.3.1 Natural seed bank

Only a small fraction of seeds collected from the soil seed bank of *Zieria prostrata* were found to be viable, with the majority being either inviable or eaten (Figure 4.6). Despite a trend towards a reduction in the number of viable seed over time (Table 4.1), this was not significant ($F_{2,8} = 1.340$, $P = 0.314$). The failure to detect any significant deterioration in seed viability over time was not a consequence of variation among plants ($F_{4,8} = 0.386$, $P = 0.8130$). There was a high degree of variability in seed bank density detected among samples, as indicated by the relatively high standard errors in Table 4.1, indicating a patchy distribution of seeds in the soil.

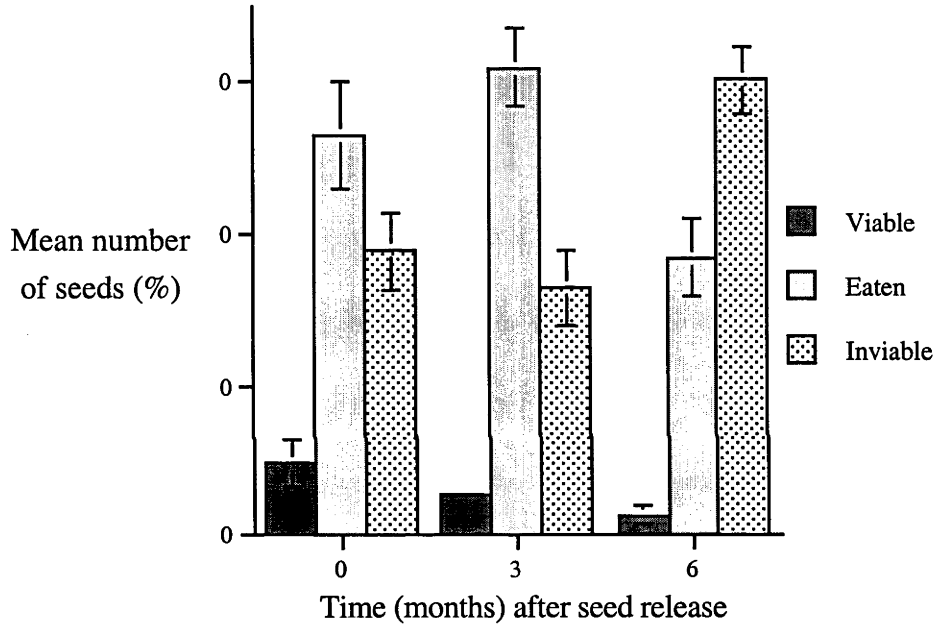

Figure 4.6 The mean percentage viability of *Zieria prostrata* seed collected at 0, 3 and 6 month intervals after seed release. Error bars represent standard error.

Table 4.1 The average size of the soil stored seed bank of *Zieria prostrata* at 0, 3 and 6 months after seed release.

Time (months) after seed release	Number of viable seeds per m^2 (mean ± SE)
0	800 ± 252
3	600 ± 306
6	180 ± 58

4.3.3.2 Seed burial experiment

Canopy cover had a significant influence upon both seed germination and seed dormancy, whereas burial depth and plot did not significantly influence either (Table 4.2). However, the influence of canopy cover was not independent of burial depth, with a significant interaction present between the two factors for both germination and dormancy. Post-hoc comparisons revealed that there were significantly more germinants and significantly less dormant seeds present when seeds were buried to a depth of only 2 cm in the disturbed plots where the canopy had been removed (D-2cm) (Figure 4.7 a and b). There was no significant difference between the remaining treatments. Therefore, the increase in germination resulting from canopy removal only occurs when seeds are buried to a depth of only 2 cm and not when buried deeper to a depth of 5 cm. The rapid increase in germination for the D-2cm treatment between 2 and 4 months corresponds to a relatively high temperature and high rainfall period (Figure 4.8). There was no obvious increase in the number of inviable seed over time (Figure 4.7 c).

Table 4.2 An analysis of variance describing the effects of various factors on (a) seed germination and (b) seed dormancy in *Zieria prostrata*.

Source	Sum of squares	Mean square estimates	Degrees of freedom	*F* ratio	*P*
a. Seed germination					
Canopy cover	2013	2013	1	14.70	0.004
Burial depth	403	403	1	2.95	0.120
Plot	288	96	3	0.70	0.574
Canopy cover*Burial depth	956	956	1	6.98	0.027
Error	1232	137	9		
b. Seed dormancy					
Canopy cover	1481	1481	1	9.47	0.013
Burial depth	386	386	1	2.47	0.151
Plot	915	305	3	1.95	0.192
Canopy cover*Burial depth	1521	1521	1	9.726	0.012
Error	1408	156	9		

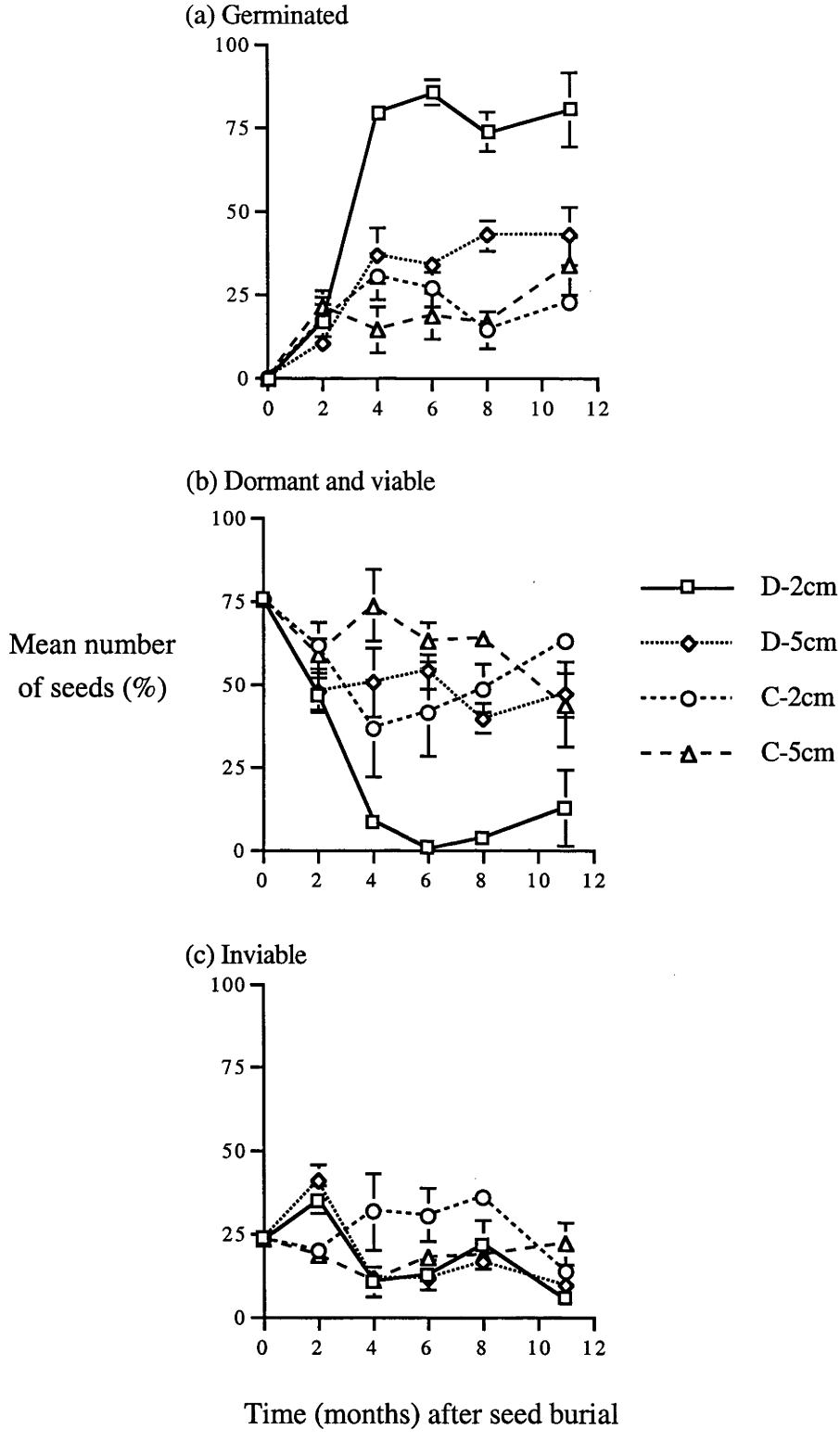

Figure 4.7 Outcomes of a seed burial experiment for *Zieria prostrata* depicting the mean percentage of (a) germinated, (b) dormant and (c) inviable seeds over an 11 month period. D-2cm represents seeds buried to a depth of 2 cm within a disturbed plot with the canopy removed, D-5cm represents seeds buried to a depth of 5 cm within a disturbed plot with canopy removed, C-2cm represents seeds buried to a depth of 2 cm under the canopy of a adult plant, and C-5cm represents seeds buried to a depth of 5 cm under the canopy of an adult plant. Error bars represent standard error.

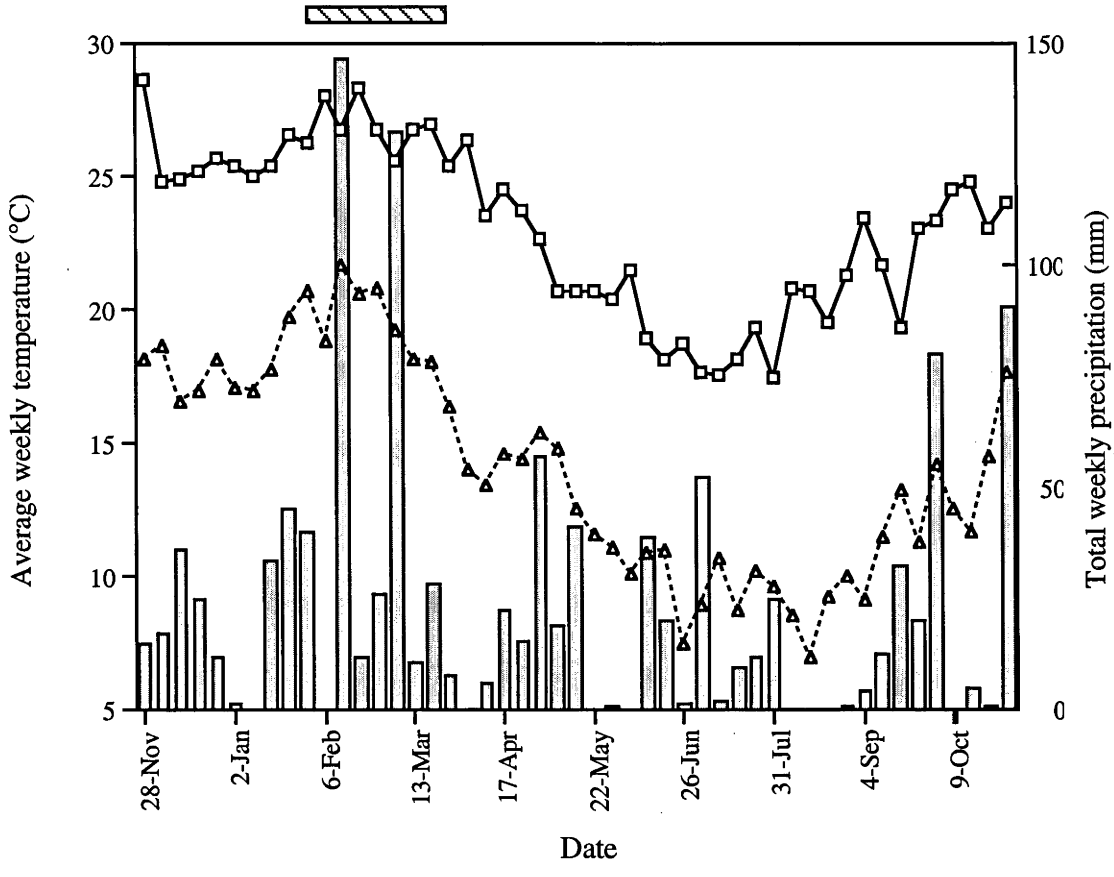

Figure 4.8 Average weekly maximum (solid line) and minimum (hatched line) temperature and weekly total precipitation, over the time period of the seed burial experiment. The bar above the graph depicts the two month period when the majority of germination occurred. Data was obtained from the Australian Bureau of Meteorology for the Coffs Harbour weather station, approximately 14 kilometres south of the study site.

4.3.4 Impact of disturbance on germination

The majority of treatment plots (13/18) revealed no seedling recruitment irrespective of treatment over the entire 12 months study period (Table 4.3). This low level of seedling emergence appears to be a consequence of low soil stored seed bank densities and/or the patchy distribution of seeds in the soil. As shown in Table 4.3, the soil seed bank densities in the subsequent season within areas adjacent to treatment sub-plots were very low or non-existent for all plots where no germinants were observed (mean of 0 to 4.5 seeds per 10 cm^2). Whereas, the seed densities were higher for those plots where germinants had been observed (mean of 1.5 to 22.5 seeds per 10 cm^2). The soil stored seed bank appears to have a very patchy distribution with viable seeds found in only nine of the 32 sub-plots sampled, with 29 viable seeds being recovered from a single sub-plot.

The analysis of variance is summarised in Table 4.4. The only factor found to influence seedling recruitment in *Z. prostrata* was disturbance, with 2.7 ± 0.56 (mean ± SE)

seedlings recorded per disturbed plot and no seedlings recorded within the undisturbed control plots. Both distance from plant (Figure 4.9) and timing of disturbance (Figure 4.10) did not have a significant impact upon seed germination. However, although not significant, there was a trend towards less germinants after the third disturbance event.

Table 4.3 Total number of *Zieria prostrata* germinants observed and average soil seed bank densities (12 months after study commenced) across all treatments in all plots of an experiment evaluating the role of disturbance in seed germination.

Plot	Number germinants	Soil seed bank densities (mean ± SE per 10 cm^2).
Site 1 plot 1	0	4.5 ± 4.5
Site 1 plot 2	2	22.5 ± 15.5
Site 1 plot 3	0	0.5 ± 0.5
Site 1 plot 4	0	0
Site 1 plot 5	0	0.5 ± 0.5
Site 1 plot 6	0	0
Site 3 plot 1	0	0
Site 3 plot 2	0	0
Site 3 plot 3	18	1.5 ± 0.5
Site 3 plot 4	25	14.5 ± 4.5
Site 3 plot 5	1	3.0 ± 1.0
Site 3 plot 6	4	1.5 ± 0.5
Site 4 plot 1	0	0
Site 4 plot 2	0	0
Site 4 plot 3	0	0
Site 4 plot 4	0	0
Site 4 plot 5	0	0
Site 4 plot 6	0	1 ± 1

Table 4.4 An analysis of variance describing the effects of various factors on seedling emergence in *Zieria prostrata.*

Source	Sum of squares	Mean square estimates	Degrees of freedom	*F* ratio	*P*
disturbance	16.9	16.9	1	8.7	0.004
time	8.6	4.3	2	2.2	0.116
distance	5.1	2.5	2	1.3	0.277
plot	17.4	4.4	4	2.2	0.072
disturbance*time	8.6	4.3	2	2.2	0.116
disturbance*distance	5.1	2.5	2	1.3	0.277
time*distance	5.3	1.3	4	0.7	0.602
disturbance*time*distance	5.3	1.3	4	0.7	0.602
error	131.7	1.9	68		

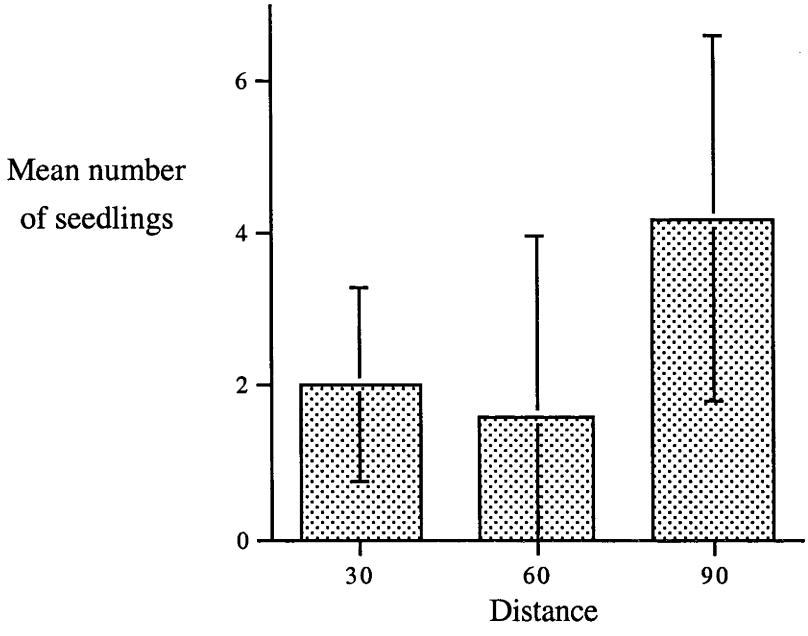

Figure 4.9 Mean number of *Zieria prostrata* seedlings emerging within disturbance plots at 30, 60 and 90 cm from adult *Z. prostrata* plants. Error bars represent standard error.

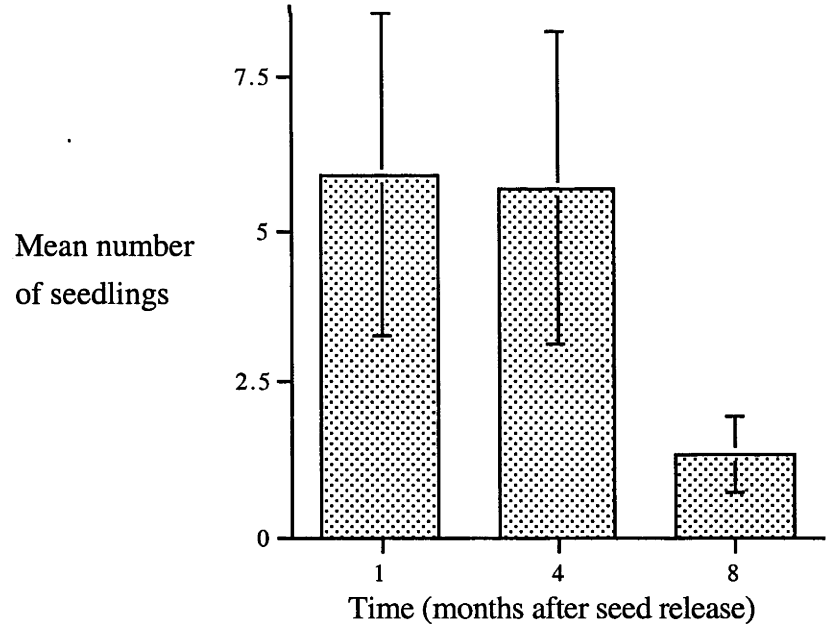

Figure 4.10 Mean *Zieria prostrata* seedling emergence within plots disturbed at various times after seed release. Error bars represent standard error.

4.3.5 Seedling survival

The number of seedlings emerging within each of the four 30 cm^2 plots was highly variable (Figure 4.11). Seedling survival decreased over time within all four plots (Figure 4.12). Of the 309 seedlings monitored, only 18 persisted beyond 16 months, and they were all restricted to only one of the four plots (plot 3). Ten months after monitoring commenced, there were no surviving seedlings within three of the four plots, despite one of these plots initially supporting approximately 250 seedlings. These three plots had been totally overgrown by the surrounding vegetation. Within plot 3, 11 of the 18 seedlings (61%) were still persisting after 16 months, with this plot still open and not overgrown by the surrounding vegetation.

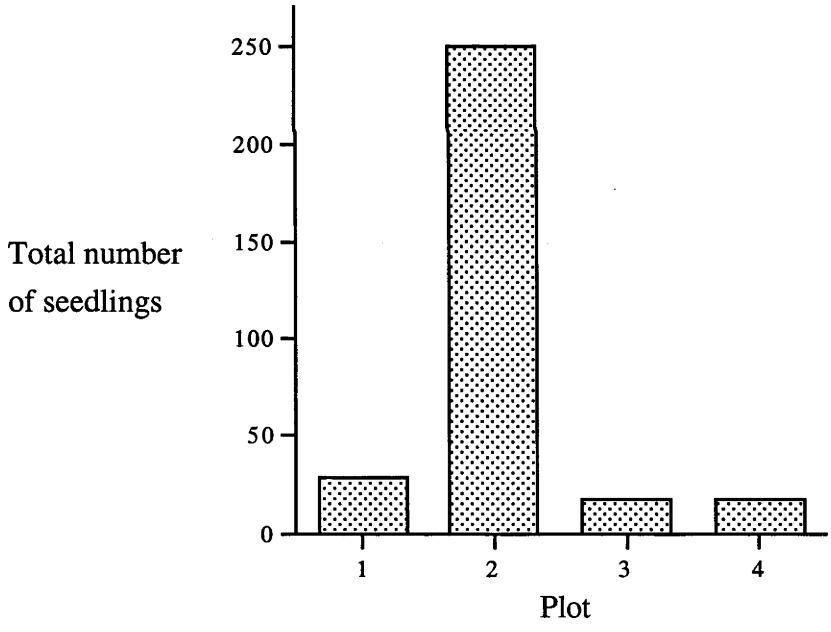

Figure 4.11 The total number of *Zieria prostrata* seedlings observed emerging within four 30 cm^2 plots after physical soil disturbance and canopy removal.

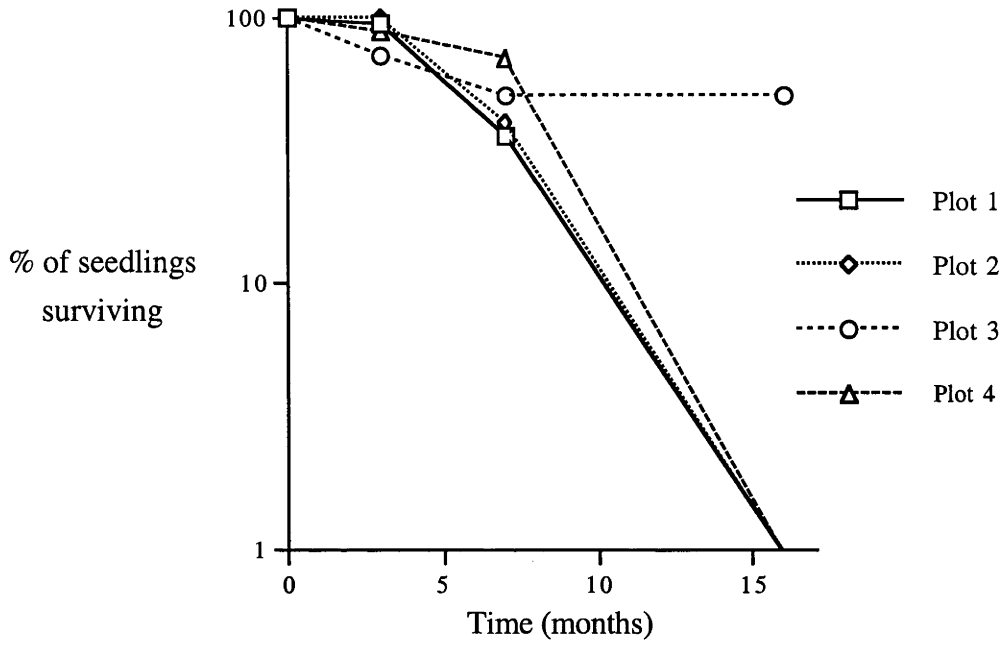

Figure 4.12 Percentage of *Zieria prostrata* seedlings surviving over a 16 month period within four 30 cm^2 plots. Note x-axis is on a log scale.

4.4 Discussion

4.4.1 General discussion of results

4.4.1.1 Seed viability and predation levels at release

A reduction in the viability of *Zieria prostrata* seeds was apparent at sites 3 and 4 over two consecutive years owing to pre-dispersal seed predation and the production of inviable seed. The identity of the seed predator is unknown, as attempts to raise the larvae were unsuccessful, however, Prakash (1995) suggested that it is likely to be a wasp larvae. The cause of the production of inviable seeds at sites 3 and 4, and not at sites 1 and 2 is unknown. Although, given the correlation between those sites exhibiting predation with those producing inviable seed, the inviable seeds may be seeds that were aborted or damaged due to unsuccessful predator attack.

The apparent absence of pre-dispersal seed predation at sites 1 and 2 is surprising given their close proximity to sites 3 and 4 (Figure 1.1, Chapter 1), where predator activity was apparent. The absence of seed predation at sites 1 and 2 may be broadly explained by two alternate hypotheses: (i) the predator does not occur at these sites, or (ii) the predator is present at these sites, but the plants avoid predator attack.

Considering the first of these hypotheses, despite the close proximity of sites 1 and 2 to sites 3 and 4, it is feasible that the predator does not occur within these populations. The

severity of seed predation has been recorded to vary strongly among habitats and along environmental gradients (Janzen 1971, 1972, 1975; Louda 1983; Louda *et al.* 1987). Perhaps the habitat at site 1 and 2 is not suitable for the survival of the predator. All four sites support a similar vegetation community, however, the environment of sites 1 and 2 is more exposed and likely to be considerably harsher, with these sites being subject to higher levels of wind and salt spray. Alternatively, perhaps the habitat is suitable, but the predator has not yet colonised these sites. It is of interest that sites 3 and 4, the only sites obviously supporting the predator, are also the only sites where translocation of *Z. prostrata* has been performed (Section 1.2). Perhaps the seed predator was introduced with the outplanting of *ex-situ* plants during the 1993 population enhancement program.

The second hypothesis, that the seed predator does occur at sites 1 and 2, but *Z. prostrata* plants at these sites avoid predator attack, would require the presence of a site specific defence mechanism such as seed toxicity. Seed toxicity is a common defence mechanism against seed predation in many plant species (Crawley 1992; Moore 1978). Cyanide, present in the form of cyanoglycosides, is one such toxin which may function in seed defence (Hendry 1993). Armstrong (ms) investigated the levels of cyanogeneic glycosides in over 30 *Zieria* taxa, many of which were found to contain high levels of this toxin, including the closely related *Zieria smithii*. Armstrong (ms) found that the level of cyanide varied considerably among *Z. smithii* individuals, with some individuals possessing no cyanogeneic glycosides and others possessing high levels. It is therefore feasible that *Z. prostrata* individuals also vary in their level of cyanogeneic glycosides, which in turn leads to differential susceptibility to predation. Despite the well documented incidences of variation in seed predation rates among individuals, the genetic basis of these differences has rarely been established (Crawley 1992). Assuming that the level of cyanogeneic glycosides is genetically controlled within *Z. prostrata* individuals, and given that sites 1 and 2 are clearly genetically isolated from sites 3 and 4 (Section 2.3.3), it is feasible that genetically determined variation in seed toxicity occurs among the sites.

In addition to the site to site variation in seed predation, predation also appeared to be plant specific. All site 3 plants possessed some eaten seeds in both 1996 and 1997. Whereas for site 4, no eaten seeds were recorded for eight plants in both years, despite being less than 100 meters from plants possessing eaten seeds. Such variation in the susceptibility of individuals to predation is expected, with the available data on pre-dispersal seed predation demonstrating that the norm is pronounced variation from individual to individual, with some plants exhibiting consistently higher rates of seed predation and others appearing to be more or less immune (Crawley 1992). For example, numerous studies have documented such variation in seed predation rates

among individual plants, or among different populations, in the same year (Augspurger 1981; Kaye 1999; Molau *et al.* 1989; Moore 1978; Platt *et al.* 1974; Solbreck and Sillen-Tullberg 1986). There are a diverse range of properties expected to vary from plant to plant which can influence the probability of predator attack or success, including: plant size, isolation, microhabitat, seed toxicity and inflorescence/infructescence size or spatial arrangements (Moore 1978). Personal observations indicate variation in predation susceptibility does not appear to be a consequence of variation in plant size or inflorescence size and/or spatial arrangement. This leaves microhabitat, isolation, and seed toxicity as possible causes of the differential susceptibility of plants to seed predation.

Microhabitat specificity of the predator may cause a plant to escape predation if it lives in an environment which the herbivore cannot tolerate (Moore 1978). The eight plants not exhibiting seed predation within site 4 were all located upon a cliff face and are therefore likely to receive greater levels of salt spray and higher wind intensity. That these plants avoided predator attack lends support to the hypothesis that the harsher environmental conditions sites 1 and 2 restrict predator activity. Isolation may be an alternate explanation for the apparent absence of predation on the cliff face plants, since isolation may reduce the probability of encounter by insects dispersing from other plants (Janzen 1968; Janzen 1970; Moore 1978). Seed toxicity may also explain differential susceptibility of individuals to predation. Given that genetic differentiation is apparent among sub-populations within site 4 (Section 2.4.1), genetically determined variation in seed toxicity is possible.

Within those sites exhibiting evidence of pre-dispersal seed predation, the level of seed predation did not vary among the two years of study. This constant level of seed predation disagrees with the available data on pre-dispersal seed predation, which demonstrate that the level of seed loss is highly variable temporally, with substantial variation in seed predation rates in the same system from year to year (Crawley 1992; Louda and Potvin 1995). So why was such temporal variation not apparent for *Zieria prostrata*? Investigating seed predation in *Z. prostrata* for only two years represents merely a snap-shot of the entire picture. Perhaps if seed predation was investigated over a longer time frame, changes in predator impact may become apparent. Pre-dispersal seed predator numbers in general, tend to be closely coupled to seed production (Crawley 1992). Year to year differences in seed production may run to several orders of magnitude and this, in turn, leads to predator satiation in the high seed years and high levels of seed predation in low seed years (Crawley 1992). Perhaps the constant predator pressure seen for *Z. prostrata* reflects constant seed production. If an especially low seed yield occurred in one year for *Z. prostrata*, the impact of predators may be

greater. For example De Steven (1983) found that fruit production was poor for two consecutive years for *Hamamelis virginana*, and in these two years, seed predation intensities averaged 80-90%. In the third year, fruit crop sizes increased dramatically, resulting in only 45% seed predation on average. Fruit production declined again in the fourth year, and the predators were able to attack nearly 100% of the available fruit. However, given that *Z. prostrata* is self-compatible and capable of autogamy (Section 4.1.3.1), extreme variation in fruit production from year to year is unlikely and thus constant predator pressure is likely to be the norm.

4.4.1.2 Seed bank dynamics

Sampling of the natural seed bank revealed that *Z. prostrata* is capable of maintaining a soil seed bank. This seed bank appears to deteriorate over time and exhibits considerable spatial heterogeneity. Sampling of the soil seed bank after the disturbance experiment also reinforced this high degree spatial heterogeneity, with viable seeds found in only eight of the 32 sub-plots sampled, and with 29 viable seeds being recovered from a single sub-plot. Spatial heterogeneity of the soil seed bank is common and may be caused by a variety of factors including rain, water run-off, wind and ants, which all act to clump seeds (Bigwood and Inouye 1988). The spatial heterogeneity of the seed bank of *Z. prostrata* may also be explained by, as outlined below, the differential rates of seed loss to germination among microhabitats.

The seed burial and retrieval experiment revealed that the longevity of the seed bank is highly dependent upon canopy cover and seed burial depth. Six months after seed burial, few dormant seeds remained when buried to a depth of only 2 cm in a canopy gap, with the majority of viable seeds lost to germination (Section 4.4.1.3). In contrast, dormant seeds remained over the entire 11 months when buried to a depth of 5 cm, or when buried beneath the canopy of an adult plant regardless of depth. Deep burial and canopy cover therefore promote the maintenance of seed dormancy and thus seed bank longevity. There was also no obvious increase in the number of inviable seeds over time irrespective of burial depth or canopy cover, implying that viable dormant seeds do not loose viability over time due to decay, at least over a 11 month period.

Dormancy prevents germination during conditions that may generally be favourable for germination, but where seedling survival is uncertain (Bell 1999; Vleeshouwers *et al.* 1995). The dormancy imposed upon deeply buried seeds and seeds under the canopy of adult plants may serve to limit competition between seedlings and adults and protects seeds from the likely failure to establish. Seeds germinating under the canopy of an adult plant would be unlikely to survive owing to competition for both biotic and abiotic resources and given the small size of *Z. prostrata* seeds, seedlings emerging from

deeply buried seeds would be unlikely to reach the soil surface (Bond *et al.* 1999). A similar promotion of seed longevity with increased burial depth was found for the three native forbs *Bulbine bulbosa, Chrysocephalum apiculatum* and *Leptorhynchos squamatus* within a closed *Themeda triandra*a grassland in south-east Australia (Lunt 1995), a habitat similar to the *Themeda* dwarf heath vegetation supporting the *Zieria prostrata* populations.

Soil seed banks can be broadly categorised according to their longevity into two main types: transient and persistent (Thompson and Grime 1979). Viable seeds fail to persist in the soil for more than one year in transient seed banks, while there is a carry-over of some viable seeds from year to year in a persistent seed bank. The longevity of the soil stored seed bank of *Zieria prostrata* will depend upon whether seeds are dispersed to a gap or under the canopy of an adult plant, and how deeply the seeds are buried. Those seeds dispersed to gaps in the canopy and not buried too deeply will form a transient seed bank, with germination occurring when the right conditions prevailed. In contrast, the majority of seeds dispersed to an area under the canopy of existing vegetation, and/or deeply, will form a persistent seed bank. Given infrequent gaps and the short distance dispersal of *Z. prostrata* seeds (Section 2.4.1), it is likely that most seeds are dispersed to areas under the canopy of existing vegetation. It is also likely that seeds will be readily buried, as they are small and compact. Therefore, these results suggest that *Z. prostrata* is capable of maintaining a persistent seed bank, with a large proportion of seeds remaining viable at least until the next seed fall.

The seed burial experiment, however, is likely to exaggerate the potential for seed persistence under natural conditions given that the seed bags remove the threat of post-dispersal predation. The incidence of post-dispersal seed predation, combined with losses to germination, may explain the observed trend towards a reduction in seed bank viability over time within the natural seed bank. The degree of post-dispersal seed predation in *Z. prostrata* is uncertain, but a preliminary post-dispersal seed predation experiment performed in 1997 suggests it may be rare, with not a single seed removed over a two day period (upub.). Nonetheless, given that the weather over these two days was overcast, it would be desirable to repeat the experiment over a longer time frame before concluding that predators do not remove *Z. prostrata* seeds.

4.4.1.3 Seed germination

4.4.1.3.1 Seed germination ex-situ

The germination trial revealed that *Zieria prostrata* seeds readily germinate, with an average of 47% of seeds germinating across all four treatments (i.e. 12h light/dark cycle with and without smoke water pre-treatment, and total darkness with and without smoke

water pre-treatment). *Zieria prostrata* seeds are therefore capable of germination in both total darkness and when exposed to light, implying that *in-situ*, seeds should be able to germinate both when buried beneath the soil and when on the soil surface. This contrasts with the outcomes of the *in-situ* seed burial experiment, which revealed that deep burial reduced seed germination (Section 4.4.1.2).

Without correcting for the number of inviable seed, both total darkness and pre-treatment with smoke water appeared to significantly increase seed germination. However, when the proportion of germinated seeds was corrected to exclude inviable seed from the total, the promotive effect of smoke water and darkness disappeared, resulting in no significant difference among treatments. It therefore appears the apparent promotive effect of smoke water and total darkness in the first instance was a consequence of the greater number of viable seeds present in the replicates pre-treated with smoke water and maintained in total darkness. This greater number of viable seeds, and hence the lower number of inviable seeds, within this treatment in comparison to the remaining treatments may be a consequence of less inviable seeds being present initially, owing to chance. Alternatively, and possibly more likely given the substantial sample sizes, the decrease in the number of inviable seed may be a consequence of smoke water minimising seed susceptibility to fungal attack and decay (Brown and van Staven 1997; Roche *et al.* 1997). If smoke water did minimise seed decay, perhaps it is the darkness that is the main germination promoter, as suggested by the earlier onset of germination for seeds maintained in total darkness (for both pre-treated with smoke water and not pre-treated) versus those maintained in a 12 h light/dark cycle (20 days versus 33 days).

The finding that smoke water did not significantly increase seed germination suggests that fire may not play an important role in the recruitment of *Z. prostrata*. Smoke water and/or aerial smoke has been found to promote germination in a diverse array of Australian plant species from fire-prone habitats (e.g. Dixon *et al.* 1995; Enright *et al.* 1997; Lloyd *et al.* 2000; Reid and Bellairs 1999; Roche *et al.* 1998; Roche *et al.* 1997). However, heat, or the interaction of heat and smoke, cannot yet be ruled out as dormancy breaking cues. The frequency of fire within the habitat of *Z. prostrata* is unknown, as large scale burning of these headlands has not been recorded. Between 1996 and 2000 there were at least three instances of small wildfires that were presumably started by arsonists. These fires did not spread beyond a few square meters. Adult *Z. prostrata* plants were killed by the fire (S. Clemesha pers. comm.) and abundant *Z. prostrata* seedlings emerged soon after (pers. obs.). It is likely the seedlings emerged following the fire in response to the resultant vegetation gap (see Section 4.4.1.3.2) rather than the properties of the fire itself.

4.4.1.3.2 Seed germination in-situ

The seed burial and disturbance experiments revealed the importance of disturbance in promoting seed germination. The seed burial experiment revealed that canopy removal enhanced seed germination of shallow buried *Z. prostrata* seeds. The disturbance experiment further reinforced the role of canopy removal on seed germination, with disturbance significantly increasing seedling recruitment.

Disturbance is known to play an important role in the dynamics of a diverse range of vegetation communities (reviewed by: Grubb 1977; Pickett 1980; Pickett and White 1985; White 1979). The importance of disturbance and gap creation in facilitating recruitment within Australian perennial grasslands, a habitat probably most analogous to that of the dwarf grassland-heathland community supporting *Z. prostrata*, is well recognised (Morgan 1997, 1998). Germination in response to disturbance has probably evolved to increase the chance of germinants surviving, given that gaps resulting from disturbance provide particularly favourable conditions for seedling persistence, owing to reduced competition for resources (Vazquez-Yanes and Orozco-Segovia 1994).

Zieria prostrata seeds are likely to have enhanced 'gap detecting mechanisms' (*Sensu* Silvertown 1980). Removal of the canopy increases both the intensity of light reaching the soil surface and the diurnal fluctuations in soil temperature and consequently alters the microclimate experienced by seeds (Morgan 1998; Thomson *et al.* 1977). It is unlikely that gap detecting in *Z. prostrata* is controlled by light, given that the laboratory germination trial found that *Z. prostrata* seeds were capable of germination when maintained in total darkness (Section 4.4.1.3.1). The gap detecting is therefore most likely related to diurnal temperature fluctuations, the ability of which to stimulate germination is well known (Ghersa *et al.* 1992; Thomson *et al.* 1977; Vazquez-Yanes and Orozco-Segovia 1994). That deeply buried seeds did not detect the gaps lends further support to temperature being the factor controlling germination, given that as depth increases, soil temperature fluctuations typically decrease (Mayer and Poljakoff-Mayber 1989).

The emergence of *Zieria prostrata* seedlings *in-situ* was not only controlled by micro-climatic conditions, but also by overall climatic conditions. The seed burial experiment revealed that the majority of *Z. prostrata* seedlings emerged within a two month period in late summer-early autumn that coincided with a relatively high temperature and high rainfall period.

Seedling emergence *in-situ* was found to be further influenced by the spatial heterogeneity of the soil stored seed bank. Seedling emergence was highly variable

among plots for the disturbance experiment, with no seedling recruitment within five of the 18 plots and for those plots where germinants arose, the number of germinants ranged from one to 25. Seedling recruitment was also highly variable within the plots disturbed for the purposes of monitoring seedling survival, with the number of seedlings emerging ranging from 17 to 250. This spatial heterogeneity of seedling recruitment is likely to be due to the spatial heterogeneity of the soil seed bank (Section 4.4.1.2).

The timing of disturbance or the distance of disturbance from an adult *Zieria prostrata* individual did not influence seedling emergence *in-situ*. However, the ability to detect any real differences between times or distances may have been minimised by the high spatial variability in seedling recruitment, particularly in terms of the influence of the timing of disturbance. There was a trend towards a reduction in seedling recruitment after the third disturbance event, seven months after seed fall, in mid-winter. This reduced recruitment may reflect inappropriate climatic conditions or seed bank depletion.

4.4.1.4 Seedling survival

Zieria prostrata Seedling survival was very low, with only 5.5% of the 309 seedlings monitored persisting beyond 16 months. Given that all surviving seedlings were located within the only plot that had not been totally overgrown by the surrounding vegetation, it appears that seedling mortality was due primarily to gap closure and presumably the consequential increased shading and competition for resources. Survival of *Z. prostrata* seedlings will therefore depend upon how long a gap remains open, and this is likely to be influenced by both gap size and the structure of the surrounding vegetation.

The impact of gap size on seedling survival in grassland communities is well documented (Goldberg and Werner 1983; Marrero-Gomez *et al.* 2000; Morgan 1997, 1998). For example, Morgan (1997) found that seedling survival in the endangered composite *Rutidosis leptorrhynchoides* was extremely sensitive to gap size, with seedling survival being restricted to large gaps of 100 cm diameter. However, given that the four gaps monitored for *Z. prostrata* survival were all of equal size (30 cm diameter), and only one gap remained open for at least 16 months, gaps closure is likely to be also influenced by the surrounding vegetation.

The majority of artificial gaps created within the habitat of *Z. prostrata* were filled primarily by canopy encroachment of adjacent plants (particularly by adult *Z. prostrata*) and by an increase in the size of adjacent plants (particularly *Themeda australis*). Seedling recruitment of other species is unlikely to play a major role in gap closure, at least in areas adjacent to adult *Z. prostrata* plants, given that very few seedlings of other

species appeared within gaps and very few seeds of other species were recovered during soil seed bank sampling. Therefore *Z. prostrata* seedling survival is likely to be influenced by the density and productivity of the surrounding vegetation. For example, Hitchmough *et al.* (1996) found that the Australian forb *Bulbine bulbosa* required competition-free gaps of 20 cm width to establish in highly productive swards, but was not gap-sensitive in low productivity swards.

4.4.2 Limits to recruitment and population stability

Limits to reproduction were found in almost every sequential step of the life cycle of *Zieria prostrata* investigated, from seed viability through to seedling survival. Seed viability was limited at sites 3 and 4 due to pre-dispersal seed predation and the production of inviable seed (Section 4.4.1.1). Soil seed bank size and longevity are unlikely to be limited, however, the spatial heterogeneity of the seed bank may limit recruitment in some instances (Section 4.4.1.2). Recruitment from the soil stored seed bank was limited by the availability of suitable micro-sites, with disturbance enhancing seed germination (Section 4.4.1.3). Finally, limits were also placed upon seedling survival, with gap closure apparently causing seedling death (Section 4.4.1.4). However, as previously mentioned, evidence of limits to a stage in the life cycle of a plant does not mean that the limiting factor(s) are limiting population recruitment, nor does a limit to recruitment mean that population stability is jeopardised (Section 4.1.2.1). Below is a discussion of the potential for each of the above factors to be limiting recruitment of *Z. prostrata*, followed by a discussion of whether limits to recruitment are likely to be limiting population stability.

4.4.2.1 Is seed viability limiting recruitment at sites 3 and 4?

Merely demonstrating an effect of predation and/or the production of inviable seed on plant reproductive output does not constitute evidence that seed availability is limiting recruitment. Indeed, most species of plants suffer seed predation rates between 30 and 100% in most years (Crawley 1983). A reduction in seed viability may not limit plant recruitment owing to either compensatory seed production or a limit to safe site availability (Andersen 1989; Crawley 1992; Hendrix 1988; Louda 1982b, 1989).

Compensatory seed production by plants in response to seed predation and/or the production of inviable seeds may lead to no reduction in the overall output of viable seeds. Plants often produce many more flowers than they could ever turn into ripened fruits. This allows substantial scope for compensating for pre-dispersal seed predation through the differential abortion of damaged fruits prior to seed fill (Crawley 1983, 1992; Marshall *et al.* 1985; Stephenson 1981).

In addition, a reduction in the production of viable seed may not decrease population recruitment because competition between individual plants for rare safe sites can cause an 'oversupply' of seeds so far as a population is concerned (Andersen 1989; Hendrix 1988). Seeds lost to predation may therefore represent 'surplus' seeds. The importance of seed losses to population recruitment at any point in time is related to the density of seeds in the soil and the abundance of safe sites (Andersen 1989; Crawley 1992).

When soil stored seed density is low or safe sites are numerous enough to be limited by seed supply, then a decrease in seed viability due to seed predation and/or seed inviability could potentially decrease population recruitment. For example, exclusion of pre-dispersal seed predators in *Haplopappus squarrosus* (Louda 1982a, 1982b), *Haplopappus venetus* (Louda 1983) and *Cirsium canescens* (Louda 1989; Louda and Potvin 1995) led to increased recruitment, demonstrating that viable seeds and not safe sites were the limiting factor, and that the insect seed predators significantly reduced population fitness.

On the other hand, if soil stored seed density is high, or safe sites for recruitment are limited, a loss of seeds to predation may lead to no measurable reduction in population recruitment (Crawley 1992). Indeed the majority of evidence suggests that seed limited recruitment is the exception rather than the rule, and that the expectation, therefore, is that seed predation will tend to have rather little impact upon plant recruitment (Crawley 1992). This expectation has been supported by numerous studies which have found that high levels of predation have not impacted upon recruitment due to safe site availability being the limiting factor (Andersen 1987, 1989; Borchert *et al.* 1989; Duggan 1985). For example Andersen (1989) investigated the impact of severe seed losses due to predation on population recruitment in four Australian species of long-lived perennials (*Eucalyptus baxteri, Leptospermum juniperinum, L. myrtinoides* and *Casuarina pusilla*). Despite insect seed predators destroying about 95% of the total seeds for each species, these losses did not have an important impact on population recruitment as effective seed supply was still very high and safe sites were limiting.

Given the potential for compensatory seed production or a limit to safe site availability, the true impact of seed losses on recruitment of *Z. prostrata* remains unknown until these seed losses are placed in the context of the overall seed dynamics of the populations. Considering the seed bank dynamics of *Z. prostrata*, that the species is capable of maintaining a persistent seed bank (Section 4.4.1.2) is likely to lessen the impact of seed predation. For example, the nearly complete herbivore destruction of the seed crop in the arid grassland shrub *Gutierrezia microcephala* in one year did not reduce recruitment the following year owing to the existence of a large seed bank

(Parker 1985). In addition, considering safe site abundance within the habitat of *Z. prostrata*, and that abundant viable seeds are still produced, it appears the impact of pre-dispersal seed predation is likely to be negated by safe site limitation (Section 4.4.2.3). Therefore it appears highly unlikely that pre-dispersal seed predation is limiting recruitment within *Z. prostrata* populations. That pre-dispersal seed predation is not limiting recruitment within sites 3 and 4 could be confirmed using either a seed addition or seed predator exclusion experiment (Crawley 1992; Louda and Potvin 1995). If neither seed addition or predator exclusion does lead to an increase in recruitment, then the availability of seed, and thus predation, does not limit recruitment.

4.4.2.2 Is seed bank size, longevity or spatial heterogeneity limiting recruitment?

It is highly unlikely that seed bank size or longevity is currently limiting recruitment within *Z. prostrata* populations, however, it is feasible that the spatial heterogeneity of the seed bank may limit recruitment in some instances. *Zieria prostrata* maintains a persistent seed bank, with seeds situated beneath the canopy of an adult plant and those buried deeply, maintaining viability for at least one year. Additionally, given that *Z. prostrata* is capable of autogamy and that abundant seed are produced each year (Section 4.1.3.1), the seed bank will receive annual inputs. However, the spatial heterogeneity of the soil seed bank (Section 4.4.1.2) may limit recruitment in some instances given that it is feasible that some disturbances will occur in sites containing few or no seeds. The extent to which such spatial heterogeneity of the seed bank limits recruitment is unknown. A seed addition experiment, (see Section 4.4.2.1), could be used to test whether seed bank size or heterogeneity is limiting recruitment. If seed addition does not lead to an increase in recruitment, then the seed bank size or heterogeneity is not limiting recruitment. Nonetheless, as with seed predation, it is likely that any limits to seed bank size or heterogeneity will be negated by limits to safe site availability (Section 4.4.2.3). However, as discussed in Section 4.4.3.2, if management removed or minimised the limit of safe site availability, then seed bank size and heterogeneity, and thus also potentially seed predation, may then become limiting factors.

4.4.2.3 Is safe site availability limiting germination and seedling survival?

It is apparent that safe site availability does limit both seed germination and seedling survival within *Z. prostrata* populations. Safe site availability was found to limit seed germination, given that disturbance enhanced seedling establishment (Section 4.4.1.3.2), and seedling survival was also found to be limited by safe site availability, given that seedlings perished when the gap that facilitated their recruitment closed (Section 4.4.1.4).

Safe sites, enabling recruitment from *Z. prostrata* seeds, are likely to only become available after adult plant death (primarily of *Z. prostrata* given restricted seed dispersal) or physical disturbance. Given the apparent longevity of *Z. prostrata* plants (Section 4.1.3.1), the availability of safe sites is most likely to be influenced by the disturbance regime. The frequency, intensity, and size of disturbance are all important components of a disturbance regime. Currently, disturbance within *Z. prostrata* habitat is likely to be low intensity and small scale, for example, eventuating from; human pedestrian activity, wind, salt spay, rock falls, water run-off, or digging by small mammals.

Unfortunately, nothing is known of the 'natural' disturbance regime of these headlands prior to human activity. Has the natural disturbance regime been altered in some form? For example, did fire once play a role in the maintenance of this community, as it does in many Australian perennial grasslands? As outlined in Section 4.4.1.3.1, large scale burning of these headlands has not been recorded. Aerial photographs of the headlands dating from the 1950's hint that, at least in recent times, disturbance may have been suppressed, with earlier photographs showing less coverage of *Banksia integrifolia.* If the natural disturbance regime of these headlands is being suppressed in some form, the structure of the vegetation community may change to a later successional stage, perhaps dominated by larger shrubs such as *Banksia integrifolia* and *Acacia sophorae*. Such a change in the structure of the surrounding vegetation would place an even greater limit upon recruitment through a further decrease in safe site availability. For example, Oostermeijer *et al.* (1994) found that the successional stage of heathlands influenced the population structure of the rare perennial *Gentiana pneumonanthe*. The young successional heathlands supported 'invasive' populations dominated by seedlings and juveniles, the stable heathlands supported 'stable' populations dominated by adults with seedlings and juveniles still occurring in high numbers, and the late successional heathlands supported 'regressive' populations comprising only adult plants.

4.4.2.4 Are limits to recruitment jeopardising population persistence?

While it is clear that safe site availability limits recruitment in *Zieria prostrata*, this does not necessarily mean that population stability or persistence is jeopardised. *Zieria prostrata* is long-lived (at least 20 years) and the adult population appears relatively stable (Section 4.1.3.1). Therefore, recruitment need not be frequent to maintain population stability. Indeed, only one new recruit would need to survive for each adult death. Given that small gaps do appear to occur naturally within the habitat of *Z. prostrata*, and given that the death of an adult *Z. prostrata* would create a gap (below which should be a large viable seed bank), these populations are most likely to be stable, at least in the immediate future. However, a longer term concern for these

populations is that a change in community structure could decrease safe site availability even further (Section 4.4.2.3). Succession towards a more aggressive closed vegetation community would not only decrease the number of gaps available for recruitment, but would also increase the rate at which gaps are closed and thus decrease the chance of seedlings persisting.

4.4.3 Conservation implications: insight into appropriate management

'Ecology becomes a predictive science when it can forecast the future-it becomes a management science when it can determine the future'
Begon *et al.* (1990) page 543.

Managers can now 'forecast', that an absence of canopy gaps within the habitat of *Zieria prostrata* will restrict safe site availability and thus limit recruitment, and conversely that an increase in the abundance of canopy gaps will increase safe site availability and thus increase recruitment. The challenge is to now use this information to 'determine', or ensure, the future of these populations.

Seed germination and seedling survival were found to be the critical life history stages limiting recruitment of *Z. prostrata* and safe site availability was found to be the limiting factor. It follows that effective management of these populations will centre upon either: (i) the maintenance of the current availability of safe sites if current population size is considered sufficient, or (ii) increasing safe site availability if an increase in population size is desired. Below I have categorised these two contrasting approaches into 'passive' and 'active' management respectively. Passive management centres upon monitoring the populations to ensure that the current vegetation community does not regress to a later successional stage, thereby decreasing safe site availability even further. Active management centres upon increasing safe site availability through physical disturbance, thereby increasing recruitment and population size.

As outlined in Section 1.2, all four populations of *Zieria prostrata* are protected within a nature reserve. For both sites 1 and 3, such reservation, when combined with population monitoring, should be sufficient to ensure the future of these populations. Within these sites, *Z. prostrata* occupies a large proportion of the available habitat area. In contrast, for sites 2 and 4, active management may be required to increase population size, thereby decreasing susceptibility to catastrophic events. As evident in Section 2.4.1, there is very limited gene flow among populations and thus the chance that *Z. prostrata* will re-colonise an area once it has been extirpated is remote.

Site 2 supports only nine *Z. prostrata* individuals and only two genotypes (Section 2.3.3), with the plants covering an area of only approximately 10 m^2 only meters from the water level. This population could therefore be easily be wiped out by a single catastrophic event such as a severe storm. Enlarging this population via active management could decrease susceptibility to such catastrophic events.

The management direction to take with site 4 is not immediately obvious. This site supports approximately 100 individuals spread across four distinct sub-populations, each typically separated by less than 100 m. Each of these sub-populations cover only a small area and are therefore susceptible to catastrophic events. Indeed, one of these sub-populations was damaged by vehicles during construction of a walkway in 1999 (pers. obs.). I failed to locate any of these plants during an inspection of the site in the following year and thus this sub-population may be extinct, at least above the ground. Additionally, the NSW National Parks and Wildlife Service has recently erected fences to exclude pedestrian activity from two of these sub-populations. This can potentially lead to excessive minimisation of disturbance and may lead to a change in the vegetation structure to a later successional stage, thereby potentially limiting recruitment. Given these concerns, management of this site may initially be passive, but quickly progress to active if any problems are identified.

4.4.3.1 Passive management

The presumably close relationship between the stability of *Z. prostrata* populations and the structure of the vegetation community (Section 4.4.2.3) has important implications for the conservation the species. It is not enough to reserve the populations and then leave them be. It is vital that any changes to both the size of *Z. prostrata* populations and the community structure are identified. Indeed, a better understanding of the relationship between plant populations and the vegetation community of which they form a part of is considered vital in the conservation and management of many threatened plant species (Hutchings 1991; Oostermeijer *et al.* 1994). It is difficult, if not impossible, to reliably identify and thus monitor individual *Z. prostrata* plants given that many intermix, forming one large prostrate mat whereby it is impossible to determine where one plant finishes and another starts. Therefore, monitoring may best be performed by monitoring the area covered by the species within permanent plots. Such monitoring would identify expansion or contraction in the coverage of *Z. prostrata* and would also allow identification of the species replacing and thus probably excluding *Z. prostrata* if restriction did occur. It would also be worthwhile to monitor any naturally created gaps to gain greater insight into the frequency of *Z. prostrata* recruitment within gaps.

4.4.3.2 Active management

Given that safe site availability is the critical factor limiting seedling emergence and survival, an increase in safe site availability and thus relaxation of this limit should lead to an increase in recruitment and thus population size. Managing vegetation by deliberately altering conditions to recruit preferred species from the seed bank has been proposed as a management tool for many threatened species (e.g. Aparicio and Guisande 1997; Oostermeijer *et al.* 1994; van der Valk and Pederson 1989).

Implementing physical disturbance that removes existing vegetation to create a canopy gap could increase safe site availability within *Zieria prostrata* habitat. So what sort of disturbance should be performed? Numerous techniques have been proposed for other species, including; small scale digging, sod cutting, grazing by sheep or cattle, mowing, and prescribed burning (Aparicio and Guisande 1997; Menges 1995; Oostermeijer *et al.* 1994). For *Z. prostrata*, any disturbance must minimise the removal of vegetation from large areas so as to prevent soil erosion, and adult *Z. prostrata* individuals should be left intact. The removal of vegetation and canopy cover from small plots adjacent to adult *Z. prostrata* plants is likely to be the most suitable technique.

Aside from the type of disturbance, there are numerous other questions relevant to using physical disturbance as a management tool. For example, how big should the gaps be? The size of gaps has been found to influence seedling survival in other species (e.g. Bullock *et al.* 1995; Goldberg and Werner 1983). For *Z. prostrata*, is there a continuous increase in probability of establishment with increasing gap size, or is there an intermediate size of opening at which establishment is maximal? Also, do seedlings benefit from maintaining the gap, and if so, how long do the gaps need to be maintained? A further question is when should the disturbance be performed? As outlined by Auld (1996), the timing of the application of a dormancy breaking cue can influence the rate of subsequent germination. The experiment investigating the impact of disturbance on *Z. prostrata* seedling recruitment found no significant difference in the rate of seed germination subsequent to three temporally distinct disturbance events, however there was a trend towards a reduction in seedling recruitment after the third disturbance event (Section 4.3.4). Given the numerous questions relating to how, at what scale, and when, disturbance should be implemented, initial disturbance trials should be set up in an experimental manner allowing further investigation of these questions. Initial experiments should perhaps be performed within the larger populations at sites 1 and 3.

One important consideration following manipulation of the habitat is that when the limits placed upon safe site availability are relaxed, the impact of limits to earlier stages

in the life cycle of *Z. prostrata* may increase. Seed bank density and heterogeneity may then become a limiting factor (Section 4.4.2.2). Indeed, that no germinants arose within 13 of the 18 experimentally disturbed sub-plots (Section 4.3.4) implies that this may be the case. In addition, if seed bank size did become a limiting factor, then it is also feasible that seed predation (Section 4.4.2.1) may in turn also limit recruitment. The initial experimental disturbance trials should therefore include comparison of the rate of germination between artificially seeded and non-seeded plots. Sowing seeds within disturbed plots could also be used to increase the area of the *Z. prostrata* population at site 4. Additionally, if disturbance trials are performed within site 4, the impact of experimentally excluding seed predators could also be investigated.

Since the enhancement of one species may result in the eventual loss of others (Franklin 1993), a further important consideration is that there are problems inherent in managing ecosystems for the benefit of a single species (Lesica and Atthowe 2000). When performing management in an attempt to increase the abundance of *Z. prostrata* it is vital that the long-term well being of other organisms in the same system and indeed the well being of the community as a whole, is taken into consideration. Managers need to be confident that actions will not negatively affect other organisms within the same system. Of particular concern is that increasing disturbance may promote invasions by non-native and weedy plant species (Eliason and Allen 1997; Hobbs 1991; Hobbs and Huenneke 1992). These concerns highlight the importance of initial experimentation prior to performing large-scale manipulations.

4.4.4 Conclusions

The primary objective of this chapter was to investigate the reproductive ecology of *Zieria prostrata* in order to, firstly, determine if there are any factors limiting recruitment, and secondly, identify management options capable of increasing recruitment if desired. Limits to reproduction were identified in almost every sequential step of the life cycle of *Z. prostrata* investigated, from seed viability through to seedling survival. However, not all these limits to reproduction are likely to limit recruitment of *Z. prostrata.* Seed germination and seedling survival were found to be the main critical life history stages limiting recruitment and safe site availability was found to be the limiting factor. It follows that effective management of these populations will centre upon either: (i) maintenance of the current availability of safe sites if current population size is considered sufficient, or (ii) increasing safe site availability via physical disturbance if an increase in population size is desired.

This research demonstrated how vital it is to investigate a species reproductive ecology prior to implementing active management. Initially, translocation with mature plants

was the proposed management option, and indeed translocation was performed at sites 3 and 4 in 1993. However, adding established individuals may have decreased short-term susceptibility to environmental stochasticity, but would not have increased long-term stability, given that safe site availability would still be the limiting factor. Therefore this research revealed a management option which is not only potentially more effective than translocation, but also less labour intensive and thus more cost efficient.

CHAPTER 5
Evaluation of the outcomes arising from research

5.1 Introduction

5.1.1 Background

In the previous three chapters I outlined research into the population genetics (Chapter 2), taxonomy (Chapter 3) and reproductive ecology (Chapter 4) of *Zieria prostrata.* All three areas of research provided conservation implications for the management of *Z. prostrata.* This study, along with the vast body of literature outlining research conducted on threatened plant species, leave no doubt that research can provide conservation implications for the management of threatened flora. On the other hand, it is not immediately obvious, from this study or the scientific literature in general, whether these conservation implications actually lead to management outcomes, or in other words, cause a change in management. Nor is it obvious whether any management outcomes lead to practical outcomes, i.e. result in an improvement in the protection and/or status of a species. For example, a literature survey[1] of studies conducted on the population genetics of rare plants (n = 141), revealed that of those studies justified solely by their potential relevance to conservation (n = 63), 90% did indeed provide conservation implications for the management of the rare plant concerned (Hogbin *et al.* 2000). It was not obvious, however, in virtually all cases whether these implications actually led to management or practical outcomes. This may be in part because these outcomes will rarely be reported in the primary literature. Alternatively, it may be because very few of these studies actually led to management or practical outcomes. Insight into the actual contribution of genetic research, or indeed any form of research, cannot be obtained without evaluating the outcomes of research already conducted.

If research is failing to lead to practical outcomes in some instances, it is due to no fault of the research itself, but rather to a failure to identify research actions that are likely to lead to practical outcomes. For example, in their review and evaluation of recovery plans written for the U.S. Fish and Wildlife Service (n = 98), Schemske *et al.* (1994) found that research into population or ecological genetics was proposed for 26 of the

[1] Publications spanned the years 1988-1998 and were collated from the following journals: American Journal of Botany, Australian Journal of Botany, Biodiversity and Conservation, Biological Conservation, Biological Journal of the Linnean Society, Canadian Journal of Botany, Conservation Biology, Evolution, Heredity, International Journal of Plant Sciences, Journal of Heredity, Molecular Ecology, Plant Systematics and Evolution, Restoration Ecology, and Systematic Botany.

species. Nevertheless, in virtually all cases it was not clear how this research would aid in recovery. I identified similar concerns after reviewing a sample of recovery plans prepared by the NSW National Parks and Wildlife Service (n = 31). Of these recovery plans, 39% proposed genetic research, however it was not obvious in 62% of these as to why genetic research was proposed. If we are to maximise the likelihood of research leading to practical outcomes for conservation, and thereby increase the effectiveness and cost efficiency of recovery programs, it is vital that thought is given to how the potential outcomes of research can assist in conservation management when prioritising research tasks both within and among species.

Before proceeding further, it is important to note that I recognise a clear distinction between research motivated by the desire for practical outcomes and research motivated by the need to experimentally tackle the many outstanding questions in conservation biology. The following approach to the critical evaluation of the role of research in the management of threatened plants is only relevant to the former, those studies motivated by the desire for practical outcomes. Frequently, such studies will be conducted on a slim budget within the context of a particular recovery program, and with the reasonable expectation of a practical outcome. Of course the later, studies motivated by the desire to gain greater insight into the many interesting research questions in conservation biology, can and do contribute much to the effective conservation of threatened plant species (Hogbin *et al.* 2000).

5.1.2 Chapter objectives

The primary objective of this chapter was to evaluate the practical outcomes arising from research into the population genetics, taxonomy and reproductive ecology of *Zieria prostrata* in order to gain greater insight into when and how research is likely to assist in conservation management.

More specifically, the objectives of this chapter were to:

1. Summarise the conservation implications arising from research into the population genetics, taxonomy and reproductive ecology of *Z. prostrata.*

2. Evaluate whether these conservation implications firstly, led to management outcomes, and secondly, led to practical outcomes.

3. Outline the lessons revealed on the role of research in the management of threatened flora.

5.2 Evaluation of outcomes

For the following evaluation of the outcomes arising from research conducted on *Zieria prostrata*, I have applied and expanded upon a framework developed by colleagues and myself specifically for the evaluation of practical outcomes arising from genetic research (Hogbin and Peakall 1999; Hogbin *et al.* 2000).

5.2.1 Possible outcomes arising from research

In order to facilitate the evaluation of the outcomes arising from research, a schematic representation of the possible outcomes is depicted in Figure 5.1. The majority of research projects are likely to provide conservation implications, however it is feasible that some research projects will not. By conservation implication, I mean a specific management recommendation. For example, a common conservation implication arising from genetic studies when population differentiation is detected, is that all populations should be protected (e.g. Cosner and Crawford 1994; Daniels *et al.* 1997; Dole and Sun 1992; Hurtrez-Bousses 1996; James and Ashburner 1997; Sydes and Peakall 1998). Conservation implications may in turn lead to a change in management (management outcome). For example, additional populations may be incorporated into the reserve system, with the final practical outcome being the adequate reservation of all populations. In other cases, conservation implications may not lead to a change in management because appropriate management is already in place, in which case the results merely reinforce the value of current management practices. For example, all populations may already be protected. Alternatively, the implications may not be relevant to the current management plan. For example, it may be that the current level of protection afforded the populations is as good as can be achieved based upon current funding or land ownership.

5.2.2 Actual outcomes arising from research

The conservation implications arising from research into the population genetics, taxonomy and reproductive ecology of *Zieria prostrata* are summarised below. These implications are then each assessed for whether they, firstly, led to management outcomes, and secondly, whether they led to practical outcomes. The assessment of each area of research has been superimposed onto the generic schematic representation of the possible outcomes arising from research (Figure 5.2).

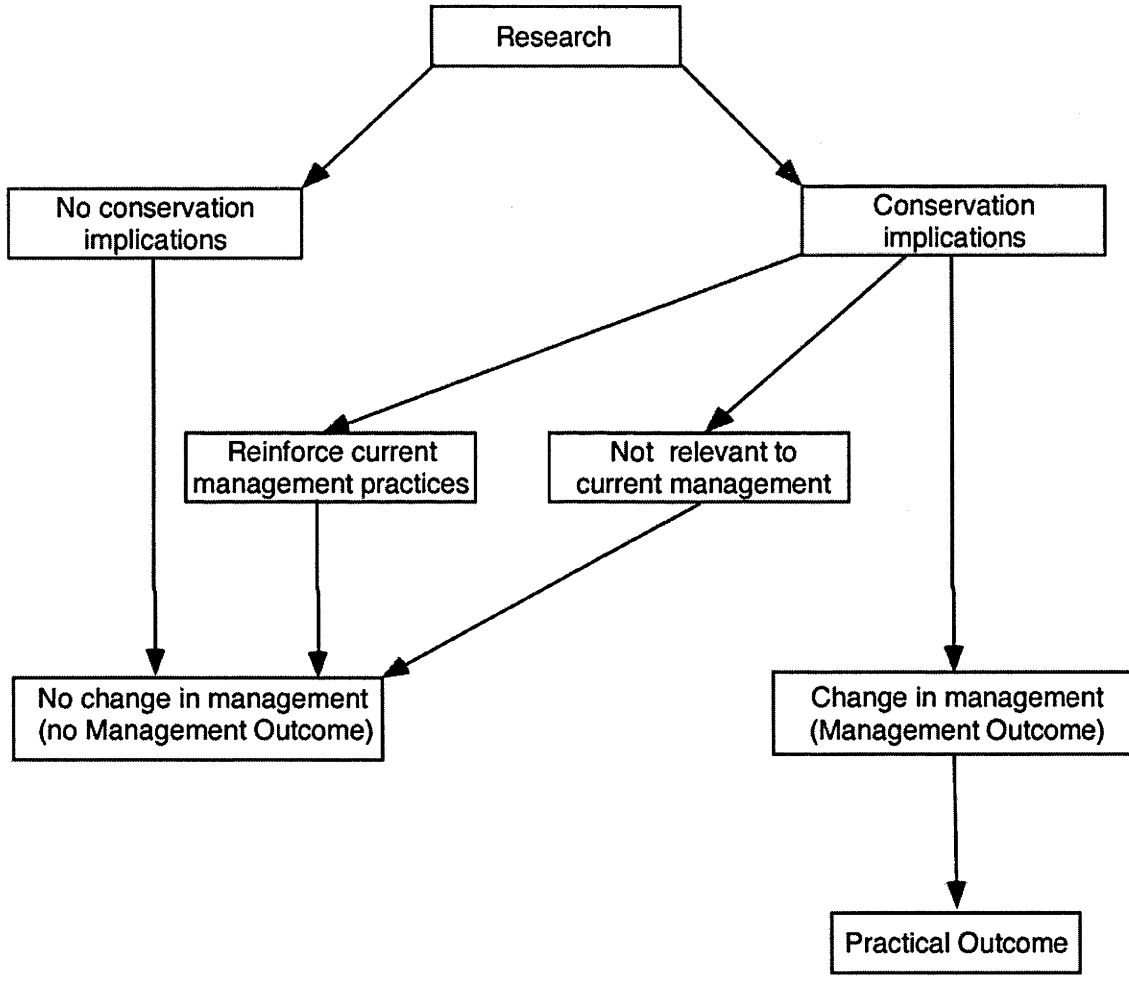

Figure 5.1 A schematic representation of the possible outcomes arising from research conducted on threatened plant species. Modified from Hogbin *et al.* (2000).

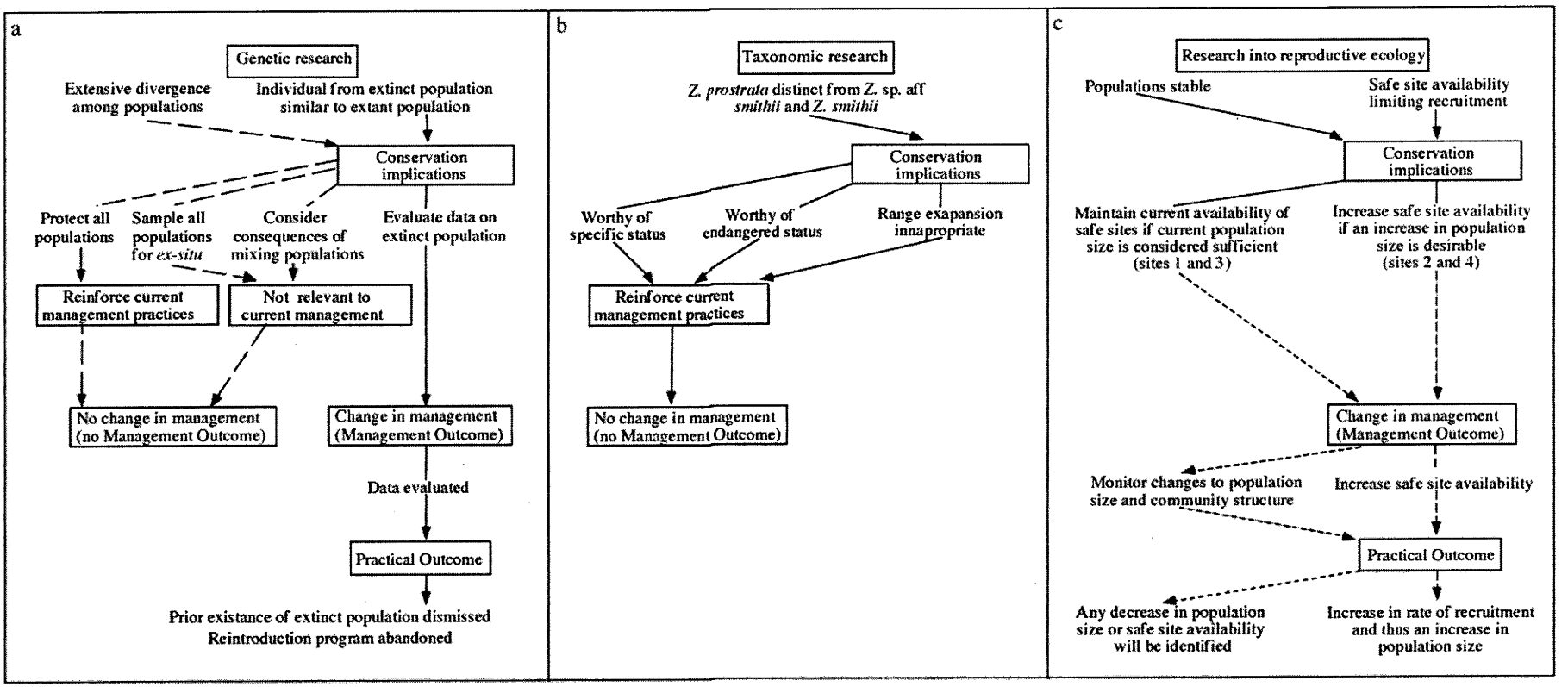

Figure 5.2 A schematic representation of the conservation implications, management outcomes and practical outcomes arising from research conducted into the (a) population genetics, (b) taxonomy and (c) reproductive ecology of *Zieria prostrata*. The hatched lines in (c) do not represent actual outcomes, but rather, speculated future management and practical outcomes.

5.2.2.1 Outcomes arising from research into population genetics

Research into the population genetics of *Z. prostrata* revealed, firstly, that extensive genetic divergence occurs among the four extant populations (Section 2.4.1), and secondly, that the individual apparently rescued from the extinct population (site 5) is genetically similar to individuals from one of the extant populations (Section 2.4.2). These two insights provided four conservation implications for the management of *Z. prostrata* (Section 2.4.3) (Figure 5.2a). First, the loss of any one population would lead to a dramatic loss of genetic variation and thus it is important that all populations be conserved. Second, an adequate *ex-situ* collection must sample the full range of genetic diversity from all populations. Third, the potential genetic consequences of mixing populations is an important consideration if further translocations are to proceed. Finally, the genetic similarity of the individual from the apparently extinct population, to individuals from one of the extant populations, raised doubts about the populations prior existence and thus led to the conservation implication that records of the extinct population needed to be evaluated.

Not all of these conservation implications have thus far led to management outcomes (Figure 5.2a). The conservation implication that all populations of *Z. prostrata* should be preserved may at first seem to be an important implication. However, given that only four populations are known, all populations require preservation irrespective of the genetic considerations. Indeed all four populations were gazetted as nature reserve in 1995, prior to completion of this study (Section 1.2). Thus, while reinforcing the importance of protecting all populations, this implication did not result in a change to management.

The conservation implication concerning the need to sample all populations in order to create and adequate *ex-situ* collection has not yet led to a management outcome as an *ex-situ* collection is not currently considered a management priority. Given that all four populations are reserved and that populations appear stable, at least in the immediate future (Section 4.4.2.4), the creation and maintenance of an *ex-situ* collection is currently considered unnecessary (NPWS 1998). Even if the creation of an *ex-situ* collection was a management priority, this implication would more than likely still fail to translate into a change in management and would merely reinforce current management practices (Hogbin and Peakall 1999). If guidelines outlining sampling strategies for *ex-situ* collections were followed (e.g. Australian Network for Plant Conservation 1997a; Botanic Gardens Conservation International 1995; Brown and Briggs 1991) is likely that an adequate representation of genetic diversity could be

achieved without genetic analysis. Because only four populations exist, it is feasible to adequately sample all populations and thus automatically capture the majority of the species' genetic diversity.

The conservation implication concerning the consequences of mixing populations has also not yet led to a management outcome given that translocation is no longer considered a management priority. Despite initial management activities focusing upon translocation (Section 1.2 and 2.1.1), insight gained into limits to the reproduction of *Z. prostrata* (Section 4.4.2) revealed that physical disturbance is a more appropriate management tool (Section 4.4.3.2).

In contrast to the previous implications, the unexpected genetic similarity of the sample reputedly rescued from the now extinct population (site 5) has already had a management outcome. This finding led to a thorough investigation of the evidence supporting the prior existence of the population (Section 2.4.2). Unexpectedly, all avenues of investigation failed to provide any convincing evidence that *Z. prostrata* grew at the site. This lead to the conclusion that the prior existence of the extinct population was doubtful and forced the abandonment of a proposed reintroduction program. This represents a major change in the management plan for the species with a clear practical outcome. Without this genetic study, further expensive and time-consuming reintroduction would have proceeded unnecessarily. Funds have now been redirected to the management of the extant populations.

5.2.2.2 Outcomes arising from taxonomic research

Research into the taxonomy of *Z. prostrata* revealed that the taxon is distinct from both *Z.* sp. aff. *smithii* and *Z. smithii* (Section 3.4.3) (Fig.5.2b). This finding has three main conservation implications (Section 3.4.4.1). Firstly, *Z. prostrata* is worthy of specific status, consistent with its existing informal status. Secondly, given confirmation of the species restricted distribution, *Z. prostrata* retains its current conservation status of endangered. Finally, as *Z. prostrata* is likely to be of recent origin and has most likely always been rare, any range expansion through translocation may be inappropriate.

In contrast to conservation implications arising from genetic research, none of the implications arising from the taxonomic research led to a change in management. All merely reinforced current management practices (Figure 5.2b). While the confirmation of the specific status of *Z. prostrata* will ultimately result in formal publication, this will not lead to a change in management given that the taxon is already widely regarded as a distinct species (Section 1.2). Similarly, that the species was confirmed to be worthy of

its endangered status merely reinforces the validity of its current status. The conservation implication that range expansion may be inappropriate merely reinforces current management given that any further attempts at range expansion through translocation were abandoned after the outcomes of the genetic research (NPWS 1998; Section 2.4.2).

However, while the conservation implications arising from the taxonomic research did not lead to a change in management in this case, this does not mean that taxonomic research was not justified. The changes to the management of *Z. prostrata* would have been drastic if either of the alternate hypotheses (Section 3.1.2) had been accepted. For example, if *Z. prostrata* had been found to be indistinguishable from *Z.* sp. aff. *smithii*, the geographic distribution of *Z. prostrata* would greatly expand from only three kilometres to over 600 kilometres. This would require an expansion of management efforts to cover the management needs of the additional populations. Indeed, given the increased geographic range, this outcome would likely change the conservation status of *Z. prostrata* from endangered to vulnerable, thus also altering the species management priority. In such a situation, perhaps any future active management actions (Section 4.4.3) would be given a low priority.

5.2.2.3 Outcomes arising from research into reproductive ecology

Research into the reproductive ecology of *Z. prostrata* yielded two important insights into the population dynamics of the species. First, populations appear to be stable, at least in the short-term (Section 4.4.2.4). Second, seed germination and seedling survival are the critical life history stages limiting recruitment, and safe site availability is the limiting factor (Section 4.4.2.3). This knowledge contributes two important conservation implications (Fig 5c) (Section 4.4.3). Firstly, to maintain current population sizes, management should centre upon maintenance of the current availability of safe sites. Secondly, to increase current population sizes so as to maintain population stability in the face of possible catastrophic events (as for sites 2 and 4), management should be active and centre upon increasing the availability of safe sites.

Importantly, this later implication does not lead to the conclusion that the more involved and costly process of translocation is necessary. The initial recovery efforts for *Zieria prostrata* concentrated upon population enhancement through translocation, however the reason for translocation was not obvious (Hogbin and Peakall 2000). The only justification for the translocation program was: '(Site 4) has suffered considerable disturbance as a result of indiscriminate vehicle usage....it is probable that *Z. prostrata* has here experienced losses. Given that the remaining population at (site 4) is extremely

small, the strategy of *in-situ* cultivation seems appropriate' (Griffith 1992). Population enhancement via the addition of adult plants was implemented at this site in 1993 before land tenure was secured, land management was implemented and apparently even before a detailed site survey was conducted (Hogbin and Peakall 2000; Section 1.2). At the same time, population enhancement also occurred at site 3, a relatively large and undisturbed population, even though this task had not been proposed in the recovery plan.

Given that the afore mentioned conservation implications have been proposed only recently, it is not yet known whether they will lead to changes in management. Nonetheless, for the long-term security of *Z. prostrata* it is crucial that the changes in management portrayed by this knowledge be put into place as soon as practical. To enable the continuation of the evaluation process, I have taken the optimistic approach of assuming these management recommendations will be heeded and have speculated as to the future management and practical outcomes that will hopefully arise from these implications (Figure 5c). In response to the first conservation implication, that to maintain current population sizes management should centre upon maintenance safe site availability, a monitoring program following both the coverage of *Z. prostrata* and the structure of the surrounding vegetation community needs to be established (Section 4.4.3.1). In response to the second implication, to increase current population sizes management should centre upon increasing the availability of safe sites, experimental disturbance trials need to be established at sites 2 and 4 (Section 4.4.3.2).

It is highly feasible that implementation of these management actions will lead to practical outcomes (Figure 5c). For example, monitoring changes to both the coverage of *Z. prostrata* and the structure of the surrounding community would identify any changes to the community structure which may decrease safe site availability. Such identification would allow responsive management in order to maintain the current availability of safe sites. Additionally, the implementation of experimental disturbance trials at sites 2 and 4 could potentially lead to an increase in recruitment and hence population size, in turn leading to an increase in population stability in the face of catastrophic events.

5.3 Generic lessons on the role of research in management

5.3.1 Not all conservation implications will lead to management outcomes

Despite the likelihood that research of any form will provide some conservation implications for the species concerned, not all implications are expected to lead to management outcomes. In the case of *Zieria prostrata*, while research into the population genetics, taxonomy and reproductive ecology of the species provided a number of conservation implications, not all these implications actually led to management outcomes. There were two main reasons why some conservation implications did not translate into management outcomes. First, similar recommendations were already in place and therefore the conservation implications merely reinforced current management. Second, conservation implications, while sound, were not relevant to current management priorities. Therefore, the management in place, and the management planned for the near future, strongly influence the likelihood of research leading to management or practical outcomes. It is necessary to note that management priorities will be influenced not only by biological factors but also external economic or political constraints. Indeed, Schemske *et al.* (1994) highlighted the importance of considering these constraints given that they may prevent practical outcomes in many instances.

5.3.2 Genetic research can be a valuable tool for confirming the composition of *ex-situ* collections

This study highlighted the value of genetic research in evaluating the genetic composition of *ex-situ* collections. The application of genetic research to the investigation of the genetic composition of *ex-situ* collections, particularly when these collections are to be used in translocation activities, or are of uncertain origin, can potentially in many instances lead to management outcomes. Such a valuable application of genetic research has seemingly only recently been recognised. For example, Gemmill *et al.* (1998), using allozyme analysis, found that the *ex-situ* collections for the two endangered plants *Brighamia insignis* and *B. rockii* were genetically representative of natural populations and hence may appropriately serve as source stock for population enhancements. On the other hand, Calero *et al.* (1999) and Ibanez *et al.* (1999) found a total absence of genetic variation within *ex-situ* collections of *Lysimachia minoricensis*, a plant now extinct in the wild. Similarly, Ricci and Eaton (1997) and Maunder *et al.* (1999) found low levels of genetic diversity in *ex-situ* collections of *Sophora toromiro*, a tree now extinct in the wild. Maunder *et al.* (1999)

were also able to identify misidentified trees of a different species using genetic evidence, prompting their subsequent removal from the collection.

5.3.3 Genetic research is not necessarily a prerequisite for the effective conservation of genetic diversity *in-situ*

Concerns about conserving genetic diversity only become relevant when all populations are not already protected or when it is not possible to conserve all extant populations adequately. This highlights a critical point that is frequently overlooked. The best way to conserve genetic diversity is to preserve all populations, in which case we do not need formal genetic studies. Indeed for many endangered plants, the number of populations is already so low that we cannot afford to loose a single population (Hogbin and Peakall 1999; Peakall and Sydes 1996). That is, ecological considerations lead to the same recommendation as genetic considerations. Thus, if all populations are protected, or is it possible to protect all populations, genetic research may not be justified, since by preserving all populations the species total genetic variability is automatically conserved (Hogbin *et al.* 2000).

If it is not possible to reserve all populations, and a choice exists as to which populations can be reserved, genetic research may provide valuable insights for reserve design (e.g. McCue *et al.* 1996; Palacios and Gonzalez-Candelas 1997a; Prober 1994; Richter *et al.* 1994). However, for genetic knowledge to actually contribute in a practical way to reserve design, procedures that allow the genetic results to be incorporated into the criteria for reserve selection must be in place. I am not aware of any study that has actually, rather than potentially, contributed to reserve design in this way. If such cases do exist, it will be of interest to determine if the genetic data actually influence the reserve design or if genetic considerations merely reinforce the preferred design. For example, ecological considerations will frequently suggest that the full range of the species should be represented in the reserve system. If this is achieved, this may adequately capture the genetic diversity without the need for formal genetic study.

5.3.4 Genetic research is not necessarily a prerequisite for the creation of a genetically representative *ex-situ* collection

If it is feasible to sample all populations, and there are no indications of clonality or other non random genetic structure, then effective sampling of genetic diversity may be achieved by random sampling of each population (Holsinger and Gottlieb 1991; Peakall and Sydes 1996). These assumptions are made in the various guidelines on *ex-situ* sampling (e.g. Australian Network for Plant Conservation 1997a; Botanic Gardens

Conservation International 1995; Brown and Briggs 1991). Therefore, genetic research is likely only to be necessary for the purposes of creating an *ex-situ* collection when there are indications of clonality or other non-random genetic structure, or if it is not feasible to sample all populations. In the later instance, if there are so many populations, it is unlikely an *ex-situ* collection is necessary.

5.3.5 Taxonomic research is crucial if taxonomic status is uncertain

If the taxonomic status of a threatened plant taxon is uncertain, and if any of the alternate taxonomic hypotheses could potentially lead to a change in management, then taxonomic research will be justified, irrespective of the eventual outcomes. However, in the unlikely scenario that none of the alternate hypotheses could potentially alter management actions, then such research may not be justified. Nonetheless, such research would clearly be of importance, but should be funded by funds not set aside for recovery actions or should be given a low priority in the recovery planning process.

5.3.6 Some form of research into demography or reproductive ecology is always justified

At least some form of demographic monitoring or basic research into reproductive ecology will always be justified for those species subject to recovery efforts, however the depth of research required will vary among species. Schemske *et al.* (1994) suggest that the most effective course of action towards the recovery of an endangered plant species requires a demographic assessment of biological status, the identification of life history stages that have greatest impact on population growth, and the determination of the biological processes affecting these stages. However, in some instances only the first step in this process will be vital. If a demographic assessment reveals a diverse age structure (i.e. the presence of seedlings, juveniles and adults of varying ages), and there is no evidence to suggest the populations are in decline, then further research into the reproductive ecology of a species may not be necessary. On the other hand, if a demographic assessment reveals, for example, a lack of seedlings or juveniles, or there is evidence that the populations are not stable, then further research is justified. This research should initially attempt to identify the life history stages that have the greatest impact on population growth, and should then focus upon determining the processes affecting these critical life history stages.

Indeed, currently, research into the demography and reproductive ecology of threatened species does seem to be receiving the attention deserved. For example Schemske *et al.* (1994) found in their review of recovery plans (Section 5.1.1) that the areas of research

that received the greatest emphasis were ecology, motivated primarily by the search for 'limiting factors' and demography, mostly involving the monitoring of individuals and/or populations. A similar pattern of emphasis upon such research is seen in the reviewed sample of NSW National Parks and Wildlife Service recovery plans (Section 5.1.1), with 88% of plans proposing some form of ecological research or demographic monitoring. Admittedly, recovery plans merely propose research, it is not immediately obvious whether such research will, or has, proceeded.

5.3.7 Critical evaluation of the need for population enhancement and the most effective methods by which this can be achieved is necessary

This study highlighted the importance of critically evaluating both the need for population enhancement and the most effective methods by which population enhancement can be achieved. Population enhancement is frequently proposed for threatened species before causes of the species decline are identified, likely threatening processes are identified and minimised, or detailed investigation of the biology of the species have been made (Hogbin and Peakall 2000). Many populations of threatened plant species may not require any form of active management for numbers to remain stable or may increase in size once threatening processes have been removed or lessened.

If, after critical evaluation, population enhancement is deemed necessary, it is vital to then determine the most effective method to achieve this goal (Hogbin and Peakall 2000). There are numerous active management options available for the population enhancement of threatened plants. Active management involves the use of manipulative techniques to enhance population size by directly influencing the survival of individual plants or a population (Section 4.4.3). At the same time these manipulative techniques in some instances have the potential to alter the genetic structure of a population and thus also it's evolutionary development. Consequently, it is important that such techniques be used in a management context only when necessary for the long-term survival of a species and that they are based upon sound scientific knowledge.

Active management options can be ranked according to level of manipulation and thus the level of potential risk to the species. It is desirable to consider the feasibility of the less evolutionary disruptive techniques first and then move onto those 'higher risk' options if required. At the lower risk end of active management there are techniques which manipulate or restore natural processes with the aim of increasing recruitment. Such techniques may include for example; hand pollination, burning, clearing, soil disturbance, or removal of grazing animals. The advantage of such techniques is that

they are cost effective, cause the least evolutionary disruption, and are more likely to stabilise a population at its natural density. At the high-risk end of active management options we have translocation. Translocation, with its associated high maintenance and high cost, may best be viewed as a last resort when all other options are deemed inappropriate or have failed.

5.4 Prioritising research tasks within and among species

Given that not all research will lead to practical outcomes (Section 5.3.1), for research to assist in the effective management of threatened species to its full potential, it is vital that research tasks are prioritised both within and among species. Prioritisation of research tasks requires the ability to identify when management outcomes are most likely. Such identification requires the determination of all the possible outcomes of research. Unfortunately, this is not an easy task given that the possible outcomes of research are influenced not only by biological factors but also by current and future management priorities and political or economic constraints. Therefore, all these factors need to be taken into account when considering the likelihood of research leading to practical outcomes. As outlined by Hogbin *et al.* (2000), this may best be achieved by considering the range of possible biological outcomes of research, and then determining how these would translate into conservation implications. Whether or not these conservation implications in turn will translate into management outcomes then needs to be evaluated. This is where the current and future management priorities and political or economic constraints need to be considered. If none of the possible outcomes lead to a potential change in management, then research may not be justified. However, if any one of the possible, but as yet unknown, outcomes will produce a change in management, research will be justified. Consideration of the lessons revealed by this study (Section 5.3) and those highlighted by Hogbin and Peakall (1999, 2000), Hogbin *et al.* (2000) and Peakall and Sydes (1996) should assist in this decision making process. By following this process of evaluating the prospects of a practical outcome, research funds can be directed towards those studies where practical outcomes are most likely. Such actions can potentially not only maximise the contribution of research to management, but can also improve the effective conservation of threatened plant species.

5.5 Conclusions

The outcomes of research into the population genetics, taxonomy and reproductive ecology of the endangered plant *Zieria prostrata* provided valuable insight into how to effectively manage this threatened species. However, the outcomes of this research can

also be applied to the effective management of other threatened plant species. Evaluation of the practical outcomes arising from this research provided a number of lessons on the role of research in the management of threatened flora. In addition, it was apparent that if research is to assist in the effective management of threatened species to its full potential, it is vital that thought is given to the possible outcomes of research while prioritising research tasks both within and among species. The identification of when research is likely to lead to practical outcomes however requires an understanding of not only the possible biological outcomes of research, but also current and future management priorities and potential political or economic constraints. Conservation biologists are clearly capable in the first of these requirements, identifying the possible biological outcomes of research, but cannot be expected to fully understand the breadth of external factors which may limit the implementation of recovery efforts. Similarly, conservation managers, or those responsible for the planning of recovery programs, cannot be expected to understand the diverse array of possible biological outcomes, but on the other hand have a better knowledge of the potential limitations external factors may impose. The resultant need to enhance communication among conservation biologists and conservation managers is well recognised (Schemske *et al.* 1994; Meffe 1998; Fleishman *et al.* 1999; Flaspohler *et al.* 2000) and the establishment of recovery teams that combine expertise from both scientific and management organisations is a move in this direction. The combination of such expertise can not only enhance the achievements of both conservation biologists and conservation managers, but will also ensure the continued preservation of many of the large number of threatened plant species currently facing extinction.

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Publications

Evaluation of the Contribution of Genetic Research to the Management of the Endangered Plant *Zieria prostrata*

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Abstract: Zieria prostrata *(Rutaceae) is known from only four headlands within a 3-km stretch of coastline in New South Wales, Australia. The species was presumed to have occurred at a headland 24 km south of its present range. We used random amplified polymorphic DNA analysis to assess patterns of genetic variation within and among the extant populations. The analysis also included an individual reputedly rescued from the now extinct population. A high level of population divergence was revealed by principal coordinate analysis and an analysis of molecular variance (AMOVA; 37% among populations). Our genetic findings provide implications for the conservation management of the species. First, the loss of any one population would lead to a severe loss of genetic variation. Second, an adequate ex situ collection must sample the full range of genetic diversity from all populations. Third, the consequences of mixing populations may be an important conservation consideration if further translocations proceed. Fourth, the individual apparently sampled prior to its population extinction is genetically similar to individuals from one of the extant sites. This degree of similarity was unexpected and, after further investigation, led to the conclusion that prior existence of the species at the site is doubtful. Subsequently, a planned reintroduction program was abandoned. So far, of these four management implications, only the last has had a direct management outcome. Those implications that failed to lead to practical management outcomes did so because the same management recommendations could be obtained without genetic research. Clearly, the challenge for more effective conservation is to identify those cases in which genetic studies are likely to produce practical outcomes for conservation managers. This may be best achieved by assessing the outcomes of genetic studies already conducted.*

Evaluación de la Contribución de la Investigación Genética en el Manejo de la Planta *Zieria prostrata* Amenazada de Extinción

Resumen: Zieria prostrata *(Rutaceae) es conocida en tan sólo cuatro cabos dentro de un estrecho de costa de tres kilómetros en New South Wales, Australia. Se suponía que la especie había ocurrido en un cabo a 24 km al sur de su rango actual. Utilizamos análisis RAPD para evaluar patrones de variación genética dentro y entre las poblaciones existentes. El análisis también incluyó un individuo supuestamente rescatado de la población ahora extinta. Un alto nivel de divergencia poblacional fue revelado por el análisis de coordenadas principales y un análisis de varianza molecular (37% entre poblaciones). Nuestros resultados genéticos proveen inferencias para el manejo de la conservación de la especie. Primero, la pérdida de cualquier población podría conducir a una pérdida severa de variación genética. Segundo, una colección adecuada ex-situ deberá muestrear el rango completo de diversidad genética de todas las poblaciones. Tercero, las consecuencias del mezclado de poblaciones podrían ser una consideración importante para la conservación si los movimientos a futuro proceden. Cuarto, el individuo aparentemente muestreado antes de la extinción de su población es genéticamente similar a los individuos de uno de los sitios existentes. Este grado de similitud fue inesparado y después de una investigación a fondo, se llegó a la conclusión de que la existencia de un*

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Paper submitted April 13, 1998; revised manuscript accepted September 16, 1998.

sitio anterior a los actuales es dudosa, subsecuentemente un programa de reintroducción fue abandonado. De estas cuatro implicaciones de manejo, sólo la existencia de un sitio anterior a los actuales es dudosa, subsecuentemente un programa de reintroducción fue abandonado. De estas cuatro implicaciones de manejo, solo la última tiene un resultado de manejo directo. Las implicaciones de conservación del estudio fallaron en conducir a salidas prácticas de manejo debido a que las mismas recomendaciones de manejo pudieron haber sido obtenidas sin una investigación genética. Claramente, el reto para una conservación más eficiente es la identificación de aquellos casos donde los estudios genéticos puedan producir salidas prácticas para los manejadores de la conservación. Esto podría ser obtenido en su mejor forma evaluando los resultados de estudios genéticos que ya han sido llevados a cabo.

Introduction

Recently, much attention has been focused on the importance of applying genetic knowledge to the design of conservation strategies for rare plants (Falk & Holsinger 1991; Fenster & Dudash 1994). For example, genetic knowledge has been applied in the design of sampling strategies for ex situ collections or reintroduction activities (Ceska et al. 1997; Wolf & Sinclair 1997), reserve design (Sampson et al. 1988; Coates & Sokolowski 1992), assessment of the conservation value or status of populations (Prober et al. 1990; Hogbin et al. 1998), and the assessment of a reintroduction program (Robichaux et al. 1997).

A key goal of conservation programs is the preservation of genetic diversity, because a loss of genetic variation is thought to reduce the ability of populations to adapt to changing environments and to survive (Frankel & Soulé 1981; Barrett & Kohn 1991; Schaal et al. 1991). Given the large and increasing number of threatened species, however, it is not possible to conduct genetic research on each of them. In Australia, for example, over 1000 plants are listed as threatened (5% of the native vascular flora; Briggs & Leigh 1996), and the resources to undertake genetic research on all these taxa are not available. To achieve effective and cost-efficient recovery of threatened plant species, we need to identify those cases in which genetic studies are likely to produce practical outcomes for conservation management. This may best be achieved by assessing and critically evaluating the outcomes of genetic studies already conducted. To our knowledge, there has been little critical evaluation of the role of genetic research in rare plant conservation management. We evaluate the contribution of a genetic study of the endangered plant *Zieria prostrata* (Rutaceae) to the management of the species.

Species Background

Zieria prostrata is a geographically restricted plant species, known from only four coastal headlands (sites 1–4) within 3 km of coastline near Coffs Harbour in northern New South Wales, Australia (Fig. 1). There are, in total, approximately 1100 individuals in the wild. Site 1 supports approximately 800 individuals, site 2 supports 9, site 3 supports 200, and site 4 supports 100 (New South Wales National Parks and Wildlife Service 1998). The species is presumed to have occurred at a disjunct fifth headland (site 5) 24 km south of its present distribution (Griffith 1992). The plant is a single-stemmed, prostrate shrub that grows on windswept headlands among dwarf heathland and low *Banksia* shrubland. Plants are typically 0.5–1 m in diameter but may grow larger, particularly in dense patches where the prostrate stems may intermix, making it difficult to identify separate individuals.

Z. prostrata is listed as an endangered species at a national and state level due to its restricted geographic range and low numbers (Endangered Species Protection

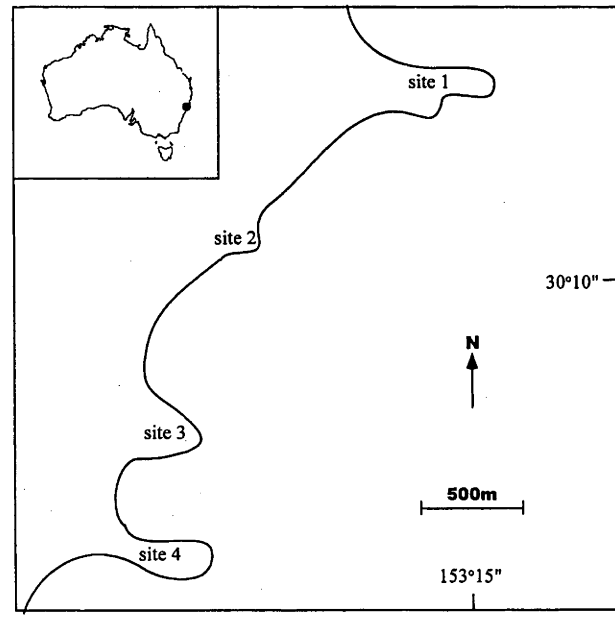

Figure 1. Geographical location of the four extant populations of Zieria prostrata.

Act 1992; Threatened Species Conservation Act 1995). The headlands upon which the species occurs have long been a popular recreational area, and vehicular and pedestrian activity is considered a major threat to this prostrate species. In 1992 a recovery plan was prepared for the species (Griffith 1992) under the Australian Nature Conservation Agency's Endangered Species Program. As part of this recovery plan an ex situ collection was established, and a subsequent population enhancement and reintroduction program was implemented in 1993. Mature plants were reintroduced into the apparently extinct, disjunct site (site 5). Stock used in this reintroduction were cultivated from cuttings of an individual apparently sampled from the site prior to extinction. Two of the extant populations (sites 3 & 4) were also enhanced with mature individuals cultivated from cuttings, with 50 plants reintroduced to site 3 and 150 to site 4 (New South Wales National Parks and Wildlife Service 1998).

Prior to continuation of the translocation program, the present genetic study was conducted to address two specific questions: (1) From a genetic perspective, does this highly endemic species consist of a single interacting population or a series of genetically isolated populations? (2) How does the genotype of the individual reputedly rescued from the now extinct, disjunct population (site 5) compare with individuals from the extant populations? It was thought that answers to these questions would assist in the development and implementation of a full-scale reintroduction program for site 5.

Methods

Fresh leaf material from all four known extant populations of *Z. prostrata* was sampled for genetic analysis. The structure of populations and differences in population size prevented comparable sampling strategies. The populations at sites 1 and 3 each form one continuous patch, and 24 individuals were sampled at 5-m intervals from a single transect at each of these sites. In contrast, site 4 contains three distinct subpopulations, each of which was represented in the total sample of 24 individuals. All 9 individuals were sampled from the smallest population at site 2. Plants known to have been planted into the populations during the enhancement program were avoided during sampling. Also, the plant reputedly rescued prior to extinction of its population (site 5) and now growing in the Coffs Harbour Botanic Gardens was sampled, as well as an individual cultivated from this source and reintroduced to the site.

Template DNA was isolated from the fresh leaf tissue as described in Huff et al. (1993) and then subjected to random amplified polymorphic DNA [RAPD] analysis (Welsh & McClelland 1990; Williams et al. 1990). The RAPD polymerase chain reaction (PCR) conditions followed those of Sydes & Peakall (1998). In an initial survey, 100 Operon decamer primers (Operon kits: OPA, OPB, OPK, OPAA, and OPAB) were evaluated for suitability. Of these, 8 primers (OPA 1, OPA 5, OPA 12, OPB 7, OPB 8, OPB 10, OPK 4, and OPK 15) were found to reveal polymorphic bands that were reproducible across multiple runs. These 8 primers were used to generate RAPD profiles for all 83 *Z. prostrata* samples.

Amplification products were resolved electrophoretically on 1% agarose, stained with ethidium bromide, and photographed on an ultraviolet transilluminator. The RAPD profile for each individual was scored directly from the photographs of the gels by assigning a value of 1 for band presence and 0 for band absence. Bands that differed significantly in frequency among populations were identified using a chi-square heterogeneity test (Zar 1984). A pairwise Euclidean distance matrix (Excoffier et al. 1992; Huff et al. 1993) was generated from the presence-absence data (RAPDistance program; Armstrong et al. 1994). The distance matrix subsequently formed the basis for examination of the patterns of genetic relationships through principal coordinates analysis (SYN-TAX program; Podani 1995). The distribution of genetic variability within and among populations and geographic regions was further investigated by an analysis of molecular variance (AMOVA) (WINAMOVA program; Excoffier et al. 1992). Site 2 was excluded from the AMOVA due to small sample size. Two separate AMOVAs were performed: the first investigated the distribution of genetic variability within and among the three populations of sites 1, 3, and 4, and the second investigated the distribution of genetic variability within and among the northern (site 1) and southern (sites 3 & 4) geographic regions. The number of permutations for significance testing was set at 1000 for all analyses.

Results

In total, the eight primers revealed 53 markers. Of these markers, 20 (37%) were polymorphic and informative. The invariant markers were excluded from further analyses. At sites 1 and 3, the RAPD profiles were highly discriminatory, with unique multilocus genotypes detected for 23 of 24 individuals at site 1 and 22 of 24 individuals at site 3. At site 2, however, only two multilocus genotypes were detected among the 9 individuals, and at site 4 only 13 out of 24 individuals had unique multilocus genotypes, with duplicated genotypes observed within all three of the subpopulations.

Duplicated multilocus genotypes are expected within a given data set when the genetic markers fail to provide sufficient resolution or when plants are unexpectedly large or clonal, or exhibit high levels of selfing. It is clearly important to differentiate among these alternatives because failure to recognize large plant size or

clonality can result in inappropriate statistical analysis of population structure. We estimated the resolving power within the data set following the methods of Sydes and Peakall (1998). The probability of identical genotypes arising via sexual reproduction was low ($0.02\text{-}2.46 \times 10^{-7}$), indicating that inadequate genetic resolution does not explain the duplicated genotypes, and therefore nonrandom reproduction (e.g., extreme inbreeding, asexuality) is the likely cause of the duplicated genotypes. Extensive examination in the field ruled out clonality and large plant size as explanations for the occurrence of duplicated genotypes (P.M.H. & R.P., personal observation). The most likely explanation for the repeated genotypes is selfing within semi-isolated subpopulations, so in the analyses we treated all individuals as genetically distinct (i.e., did not pool repeated genotypes).

Chi-square heterogeneity testing revealed that, of the 20 polymorphic markers, 13 (65%) were significantly heterogeneous across populations ($p < 0.05$), three of which were population-specific for site 2. Divergence among populations can therefore be attributed to different marker frequencies, and for site 2 also to fixed differences. The relationship between populations can be summarized by means of ordination by principal coordinate analysis (PCA), which accounted for 47.52% of the total variation in the first two axes (Fig. 2). This analysis revealed that the two multilocus genotypes that were detected in the smallest population at site 2 were clearly distinct from the remaining three populations. Noticeable population clustering corresponding to geographic relationships was apparent among the three remaining populations. Site 1, the northernmost population, formed a distinct cluster separate from the two southern populations (sites 3 & 4), whose genotypes overlap slightly in the PCA ordination space. Of particular interest is the position of the individual apparently sampled from site 5 prior to the population's extinction, which clusters close to individuals from site 3.

An AMOVA treating all three populations separately (Table 1) revealed that 37% of the total variation occurred among populations and 63% occurred among individuals within populations. Given the overlap apparent among sites 3 and 4 in the ordination space (Fig. 2), an AMOVA was also performed to compare the northern

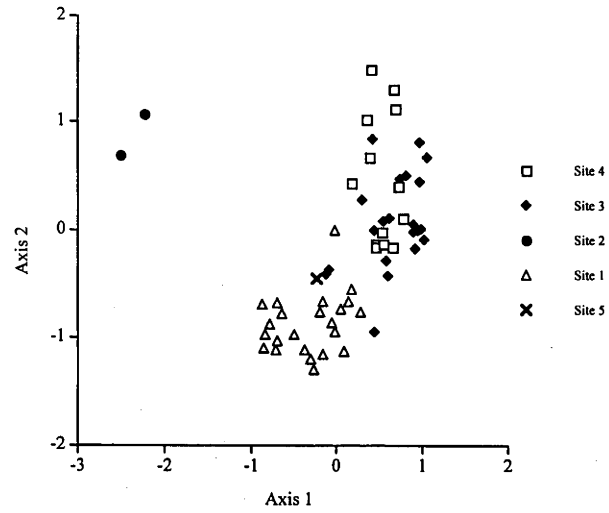

Figure 2. Principal coordinate analysis of the 84 Zieria prostrata *individuals sampled, including the four extant sites (sites 1-4) and an individual from the extinct site 5. Axis 1 extracted 30.02% of the variance, and axis 2 extracted 17.5% of the variance.*

population (site 1) with the combined southern populations (sites 3 & 4). Similar to the previous analysis, 35% of the total variation was distributed among the northern and southern populations, and 65% occurred among individuals within geographic regions. Thus the large population divergence exhibited within this species is attributable mainly to divergence among the northern and southern populations. This pattern of extensive population divergence remained when duplicated multilocus genotypes were excluded from the analyses: 35% of the variation was represented among the three populations and 33% among the northern and southern sites (full AMOVA table not shown).

Discussion

Population Genetic Structure

The four extant populations of *Z. prostrata* were highly divergent, with the smallest population (site 2) being

Table 1. Analysis of molecular variance (AMOVA) within and among *Zieria prostrata* populations[a] and within and among geographic regions.[b]

Source of variation	*df*	*SSD*	*MSD*	*Variance component*[c]	*Total variance (%)*
Among populations	2	57.8	28.9	1.12	37
Among individuals within populations	69	132.4	1.92	1.92	63
Among geographic regions	1	39.9	39.39	1.16	35
Within geographic regions	70	150.8	2.15	2.15	65

[a] *Sites 1, 3, and 4.*
[b] *Northern, site 1 and southern, sites 3 and 4. Statistics include the degrees of freedom (df), sums of squared deviations (SSDs), mean squared deviations (MSDs), variance component estimates, and the percentages of the total variance contributed by each component.*
[c] *The probability of obtaining a more extreme component estimate by chance alone is less than 0.0010.*

the most divergent, although it is geographically intermediate. The high level of divergence among populations is unexpected given that the species' range extends only 3 km. Table 2 lists the results of several previous genetic studies utilizing RAPD markers to investigate the distribution of genetic variation within and among populations. The majority of these previous studies revealed variation among populations to be less than 27%, despite the maximum distance among sampled populations ranging from 20 to 540 km. In contrast, in *Z. prostrata* the variation among populations was 37%. Such extreme divergence, given the small geographic scale (3 km), may be explained by several factors including the species' breeding system, genetic isolation of populations and/or genetic drift.

The existence of a close association between breeding system and the distribution of genetic variation is well known (Loveless & Hamrick 1984; Hamrick & Godt 1989; Hamrick et al. 1991). The extent and distribution of allozyme diversity within plants conforms more or less to one of two common patterns. In predominant outcrossers, on average only 20% of the species' genetic diversity is represented among populations, with the remainder existing as variation among individuals within populations. In contrast, in predominant selfers 50% of the genetic variability is found among populations (Hamrick et al. 1991). The level and partitioning of RAPD variation has been examined in both outcrossing and selfing plants, and patterns similar to those found for allozymes have been reported, although more studies are needed. For example, in Table 2 all outcrossing species exhibit low levels of variation among populations, ranging from 0% for *Banksia cuneata* to 27.1% for *Buchloe dactyloides*. The selfing species *Hordeum spontaneum* exhibits 43% variation among populations. *Zieria prostrata*, with 37% variation detected among populations, appears to be characterized by more extensive genetic differentiation among populations than is typical for outcrossing species. These results may imply greater levels of selfing than outcrossing for *Z. prostrata* or that mating among close relatives is occurring. Bagging experiments on plants in cultivation have revealed that *Z. prostrata* is capable of autogamy and high levels of seed set in the field, despite a lack of obvious pollinators, which suggests that self-pollination is likely (P.M.H., unpublished data).

Genetic differentiation among *Z. prostrata* populations demonstrates genetic isolation of the populations, either current or previous. Gene flow between the headlands may be minimal or nonexistent due to restricted pollen and seed dispersal. Seed-dispersal experiments have shown that *Z. prostrata* seeds disperse ballistically to a maximum distance of only 1.5 m (mean ± SE = 42 ± 3.8 cm) and appear rarely to be dispersed secondarily (P.M.H., unpublished data). Observations of seedling emergence in the field also support short-distance dispersal, with all observed seedlings located within 30 cm of an adult plant. Pollen flow is also likely to be restricted in *Z. prostrata* as a result of the species' ability to self-pollinate and the apparent lack of pollinator activity. The increasing genetic distance with geographic distance among populations of *Z. prostrata* (with the exception of site 2) also supports restricted gene flow between the headlands.

When populations are small and isolated from one another, genetic drift will also influence genetic structure and increase differentiation among populations (Barrett & Kohn 1991; Ellstrand & Elam 1993). Thus, the divergence among *Z. prostrata* populations may be further accentuated by genetic drift due to their small population size. This may explain why the small population at site 2 is extremely divergent from the remaining three populations, even though it is geographically intermediate.

The PCA clustering of the individual from site 5 with individuals from site 3 was unexpected. Given that extensive genetic divergence occurs among populations within this species across a geographic range of only 3 km, it was expected that a population 24 km south would show considerable divergence from the other populations, perhaps similar to or greater than that found for individuals from site 2.

Initially we thought that the genetic similarity of the site 5 individual to individuals from site 3 might be explained by a sampling or labeling error in the ex situ collection. We thus included in the genetic analysis the "source" plant growing in the botanic gardens along with the reintroduced individual. Because both individuals showed the same genotype, a labeling or sampling error during the reintroduction phase could be ruled out. This led us to investigate all of the available information

Table 2. The levels of genetic variation among populations of various plant taxa as determined by random amplified polymorphic DNA analysis.

Species	*Variance among populations (%)*	*Maximum distance among populations (km)*	*Breeding system*	*Reference*
Banksia cuneata	−0	195	outcross	Maguire & Sedgley 1997
Grevillea scapigera	13	540	outcross	Rossetto et al. 1995
Grevillea barklyana	13	20	outcross	Hogbin et al. 1998
Buchloe dactyloides	19.5-27.1	70	outcross	Huff et al. 1993
Eucalyptus globulis	5.1-26.2	150	outcross	Nesbitt et al. 1995
Zieria prostrata	37	3	selfing?	this study
Hordeum spontaneum	43	250	selfing	Dawson et al. 1993

on the extinct population, including searches for herbarium and cultivation records reputedly taken prior to the population extinction, and to interview those people associated with the original collections. All avenues of investigation failed to provide any convincing evidence that *Z. prostrata* grew at site 5, and we now believe that *Z. prostrata* did not occur at site 5. We suggest that the plant reputedly collected from site 5 was collected from site 3 but incorrectly ascribed to site 5. Subsequent searches at site 5 failed to find any trace of the plant, probably leading to the claim of extinction, which in turn provided motivation for an entire reintroduction program. In the current recovery plan for *Z. prostrata*, it is now concluded that reports of the species occurrence at site 5 are doubtful, and the planned reintroduction program has been abandoned (New South Wales National Parks and Wildlife Service 1998).

This case provides an example of how a lack of careful documentation and validation can ultimately and unintentionally lead to misinformation. This case also provides an example of how genetic markers can be used as diagnostic tools to identify the origin of individuals within ex situ collections or natural populations. Such use of genetic markers appears to be largely overlooked, despite its potential importance in practical conservation management. For example, Ricci and Eaton (1997), using allozymes, showed that all cultivated *Sophora toromiro* were most likely descended from a single tree, and thus recognized the need to increase variability within the reintroduced populations. Using allozymes, Reisberg et al. (1989) found evidence of hybridization within natural populations of the island endemic *Cercocarpus traskiae.* This led to the formulation of a number of management recommendations directed toward decreasing the chance of further hybridization.

Conservation Management Implications and their Critical Evaluation

Genetic studies of rare plants can provide valuable insights into the patterns and extent of genetic diversity, which in turn can provide clues about the biology and evolutionary history of a species. Understanding the patterns of genetic diversity is also relevant to conservation and can provide implications for the management of a species. For example, genetic studies may provide answers to questions such as the following: How many, or which, populations need to be sampled in order to obtain a representative ex situ collection? Is it important that all populations be protected in order to conserve total genetic variability? Are any populations genetically depauperate and thus possible candidates for active management? It should be recognized, however, that not all management implications revealed by genetic studies will actually lead to a change in management or have direct management outcomes (Mistretta 1994; Peakall & Sydes 1996). Because resources are inadequate to fund all requested genetic studies, it is essential that those studies with direct application to current conservation management be given the highest priority by conservation agencies. The challenge is to identify those cases in which genetic studies are likely to produce practical outcomes for conservation managers (Peakall & Sydes 1996). This may be best achieved by assessing the outcomes of genetic studies already conducted. Our close involvement with the development and implementation of the recovery plan for *Z. prostrata* has enabled us to critically evaluate the practical outcomes of our genetic study. Below we identify a range of conservation management implications arising from our genetic study of *Z. prostrata* and assess whether they led to practical management outcomes.

The extensive genetic divergence detected among *Z. prostrata* populations has a number of implications for management of the species. First, the loss of any one population would lead to a dramatic loss of genetic variation, so it is important that all populations be conserved. Second, an adequate ex situ collection must sample the full range of genetic diversity from all populations. In this respect it appears that the original ex situ collection was inadequate because it was based on just a few individuals from two of the populations (S. Clemesha, personal communication). Finally, the potential genetic consequences of mixing populations may also be an important conservation consideration if further translocations are to proceed. The mixing of genetically distinct populations may give rise to outbreeding depression, whereby a reduction in fitness arises due to a loss of local adaptation or break-up of coadapted gene complexes (Templeton 1986).

The management implication that all populations of *Z. prostrata* should be preserved may at first seem to be an important practical outcome of this genetic study. Given that only four populations are known, however, all populations require preservation irrespective of genetic considerations. All four populations were assigned protection in 1995, prior to completion of this study. Concerns about conserving genetic diversity become relevant only when it is not possible to conserve all extant populations adequately. In this context, our genetic study merely reinforces the importance of the prior reservation of the populations and does not indicate the need for a change of management. This case highlights a critical point that is frequently overlooked. The best way to conserve genetic diversity is to preserve all populations, in which case we do not need formal genetic studies. For many endangered plants, the number of populations is already so low that we cannot afford to loose a single population.

The initial small sample size and lack of representation of all populations in the ex situ collection suggest that further sampling is required. Although our genetic findings provide the basis for a more effective sampling of the genetic diversity of the species, it is possible that, if

guidelines outlining sampling strategies for ex situ collections had been followed (e.g., Brown & Briggs 1991; Botanic Gardens Conservation International 1995; Australian Network for Plant Conservation 1997), a more appropriate representation of genetic diversity would have been achieved without formal genetic analysis. Because only four populations exist, it is feasible to adequately sample all populations and thus automatically capture the majority of the species' genetic diversity.

The implication that the potential genetic consequences of mixing populations may be an important conservation consideration has not yet led to practical management outcomes for two reasons. First, irrespective of our genetic results or even without formal genetic study, the potential consequences of mixing populations should always be an important consideration. Our results suggest that it is important that population distinctiveness be retained for the divergent sites 1 and 2, whereas this may not be as important for the less divergent sites 3 and 4. But, this prescription assumes that neutral genetic marker variability is correlated with adaptive variability, which may not be the case (Milligan et al. 1994). Even in the absence of detectable divergence, there may be population differentiation in other traits, particularly quantitative traits that may be more closely linked to fitness (Storfer 1996). Thus, irrespective of our genetic results, the most conservative approach may be to retain, if possible, population distinctiveness. Second, direct management outcomes from this genetic implication will not occur unless translocation is required. In a parallel ecological study, we have identified a number of more cost-effective and lower-risk alternatives to translocation, including exclusion of seed predators to enhance viable seed production and soil disturbance to enhance germination. These options are favored over translocation in the present recovery plan (New South Wales National Parks and Wildlife Service 1998).

In contrast to the previous implications, dismissal of the prior existence of the fifth population of *Z. prostrata* (site 5) has already had a direct management outcome: abandonment of a major reintroduction program. This represents a significant change in the management plan for the species, with significant cost reductions, and emphasizes the importance of using genetic studies to clarify the source of ex situ collections of uncertain origin. Without this genetic study, further expensive and time consuming reintroductions would have proceeded. Even so, one could argue that this genetic study was not necessary to uncover the problem. Merely reevaluating the evidence for the previous existence of the population would have resulted in the same outcome. Nevertheless, in this case genetics did provide the impetus for the reevaluation because it raised doubts about previously assumed "facts" that otherwise would not have been disputed.

A distinction can be made between management implications that have immediate practical outcomes and those that either may become relevant to future management or simply contribute to a growing and important body of knowledge. Our genetic study provided a number of implications for the management of *Z. prostrata*, but only one of these implications actually led to a practical management outcome. Those implications that failed to lead to practical management outcomes did so because the same management recommendations could be obtained without formal genetic study.

The likelihood of a genetic study leading to practical management outcomes can be assessed by addressing specific questions in the context of the management options available for the species. This process may reveal that questions often perceived to require genetic knowledge may in fact be answered without formal genetic study. To illustrate, consider the need to design a sampling strategy for ex situ conservation with the goal of representing the species' full range of genetic diversity in the collection. As noted, it is not always necessary to understand a species' genetic structure in order to devise a suitable sampling strategy. For example, if it is feasible to sample all populations and there are no indications of clonality or other nonrandom genetic structures, then effective sampling of genetic diversity may be achieved by random sampling of each population. On the other hand, if clonality is possible, genetic study may be critical to achieving a representative collection (Sydes & Peakall 1998). Another illustration is the need to design an appropriate reserve system: if all populations are protected or it is possible to protect all populations, genetic study may not be justified because when all populations are preserved the species' total genetic variability is automatically conserved. If it is not possible to protect all populations and a choice must be made as to which populations can or cannot be protected, genetic research may provide valuable guidelines for this selection. A further priority for genetic study may be the clarification of taxonomic status when morphological data are insufficient for taxonomic resolution (Peakall & Sydes 1996).

Our study has highlighted the practical value of genetic research for clarifying the origin of an ex situ collection whose history is poorly known or uncertain. This study also demonstrates the importance of critically evaluating the need for genetic studies on a case-by-case basis. By directing funds for genetic studies to those cases in which practical outcomes are likely, the practical value of genetics in rare plant conservation can be maximized. We hope our study will motivate further critical evaluation of the role that genetics can (and cannot) play in the conservation of endangered species.

Acknowledgments

This study was completed while P.M.H. was a recipient of an Australian Postgraduate Research Award (Indus-

try). Additional funding was provided by the New South Wales National Parks and Wildlife Service and the Australian National University. We thank members of the *Z. prostrata* recovery team, in particular T. Auld, S. Clemesha, and A. Steed for their input into the project. We also thank M. Sydes, M. Ramsey, S. Krauss, and assigning editor K. Holsinger for commenting on the manuscript.

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Aust. J. Bot., 2000, **48**, 375–382

Achieving practical outcomes from genetic studies of rare Australian plants

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Abstract. An increasing number of genetic studies of rare plants are motivated by the potential contribution they can make to the conservation of the species concerned. However, while these studies frequently identify conservation implications, few have demonstrated practical conservation outcomes. In this paper, we critically evaluate the practical outcomes of our genetic study of three endangered plant species: *Haloragodendron lucasii*, *Zieria prostrata* and *Wollemia nobilis*. Each genetic study provided a number of conservation implications or management recommendations for the species concerned. However, for three reasons, not all of the implications actually led to practical outcomes. First, similar recommendations were already in place. Second, management recommendations, while sound, were not relevant to the current management priorities. Third, irrespective of the genetic outcome, a given recommendation prevailed as the preferred management option. In order to achieve effective and cost-efficient recovery of threatened flora, we need to identify those cases where genetic studies are likely to produce practical outcomes for conservation management. This may best be achieved by first, assessing and critically evaluating the outcomes of genetic research already conducted, and second, evaluating the potential for practical outcomes of future research by considering the range of possible outcomes in the context of the management options available for the species.

Introduction

The conservation of threatened plants has become an international imperative, with nearly 34 000 species, or roughly 12.5% of the world's vascular flora, facing extinction (Walter and Gillett 1998). Indeed, within Australia we are equally concerned, with 74 species already driven to extinction and over 1000 threatened species heading that way if no action is taken to reverse their decline (Briggs and Leigh 1996). The effective conservation of the threatened flora is therefore an urgent priority.

One goal of conservation is the preservation of genetic diversity, as a loss of genetic variation is thought to reduce the ability of populations to adapt to changing environments and to survive (Frankel and Soulé 1981; Barrett and Kohn 1991; Schaal *et al.* 1991). The concern for the loss of genetic diversity has led to an increase in the application of genetic knowledge to the design of conservation strategies for rare plants (Falk and Holsinger 1991; Fenster and Dudash 1994). To illustrate, a survey of genetic studies published over the last 10 years reveals that there has been a growing interest in the genetic study of rare plants (Fig. 1). Furthermore, these studies are increasingly using PCR-based DNA markers that, in general, increase the cost of study. Given the large and increasing number of threatened species, it is neither possible, nor practical, to conduct genetic research on all threatened species. Therefore, in order to achieve effective and cost-efficient recovery of threatened plant species we need to identify those cases where genetic studies are likely to produce practical outcomes for conservation management. This may be achieved best by assessing and critically evaluating the outcomes of genetic research already conducted. There has been little emphasis on the evaluation of genetic studies in this way. Yet, it is clear that we can learn much from the many existing studies, both in terms of the general patterns revealed, and the practical value of such studies.

At face value, genetic research does not appear to produce practical outcomes for conservation managers. The aforementioned literature revealed that, of those studies justified solely by the potential relevance to conservation, 90% did indeed provide implications for the management of the rare plant concerned. However, we know little about whether these implications actually led to practical management outcomes. This may be in part because these outcomes will rarely be reported in the primary literature. However, the evaluation of the practical outcomes of genetic research already conducted is surely a necessary prerequisite for maximising the future contribution of genetic research to rare plant management.

In this paper, we critically evaluate the practical outcomes of the genetic studies of three plant species: *Haloragodendron lucasii*, *Zieria prostrata* and the recently discovered relictual pine, *Wollemia nobilis*. Our close involvement with the devel-

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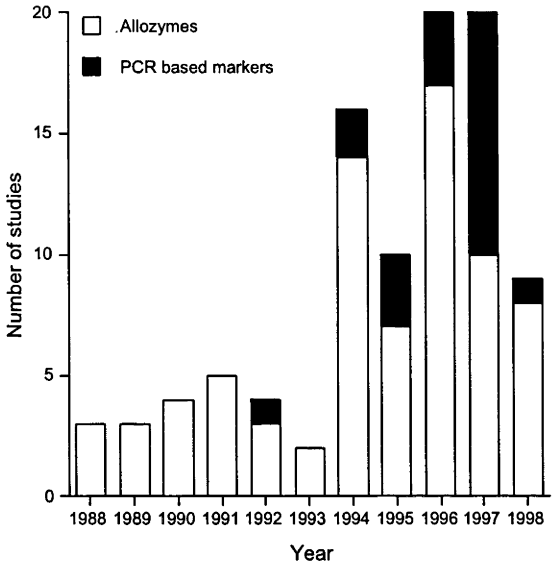

Fig. 1. The number of genetic studies of rare plants published over the past 10 years, on the basis of literature survey of 15 journals over the period of January 1988–July 1998 (see Appendix for the list of journals). Studies have been categorised into those utilising allozymes and those utilising PCR-based markers (e.g. RAPDs, AFLPs, RFLP, rDNA).

opment and implementation of the recovery plans for each of these species has made this possible.

To help facilitate our evaluation of the contribution of genetic research to rare plant management, a schematic representation of the possible outcomes are depicted in Fig. 2. Note first that not all genetic studies will provide conservation implications for the management of the species concerned. By conservation implications we mean a specific management recommendation, and we will use these terms interchangeably. For example, a common suggestion arising from genetic studies is the recommendation that all populations should be protected (e.g. Kress *et al.* 1994; Daniels *et al.* 1997; Ge *et al.* 1998). This management recommendation may lead to a change in management (management outcome). For example, additional populations may be incorporated into the reserve system, with the final practical outcome being the adequate reservation of all populations. In other cases, conservation implications may not lead to a change in management, because appropriate management is already in place, in which case the results merely reinforce the value of the current management practices. Alternatively, the implications may not be relevant to the current management plan.

Below we identify a range of conservation implications arising from our genetic studies and assess whether they led

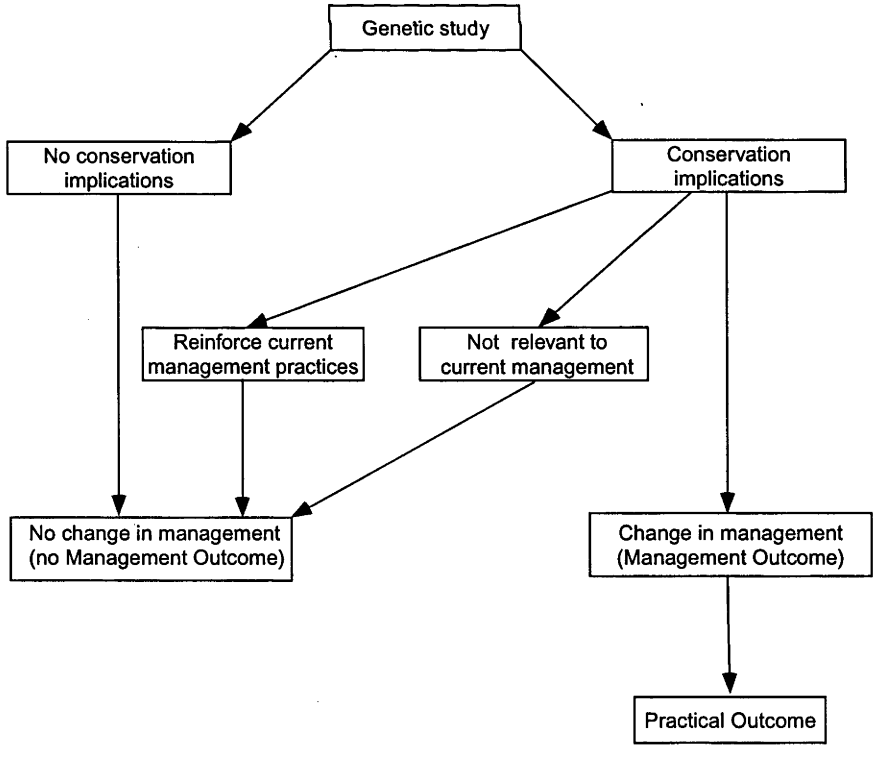

Fig. 2. A schematic representation of the possible outcomes from the genetic study of rare plants.

(1) to management outcomes and (2) to practical outcomes. In each instance, we have superimposed our evaluation onto our generic representation of the possible outcomes from the genetic study of rare plants (Fig. 3). We have avoided detail on the methods and results since these have been, or will be, published elsewhere (Sydes and Peakall 1998; Hogbin and Peakall 1999), and instead we concentrate here on evaluation of the conservation implications.

Case studies

Haloragodendron lucasii (Sydes and Peakall 1998)

The herbaceous shrub *Haloragodendron lucasii* (Haloragaceae) was presumed extinct until its rediscovery in 1986. The species is presently known from only eight sites spanning a 10-km range, near Sydney, New South Wales (NSW), Australia (only four sites were known at the start of the study in 1996). Clonality was suspected for this species and determination of the extent of clonality was one of the main research objectives of the species' recovery plan (Nash and Matthes 1994). The sprawling, dense nature of this species made it impossible to estimate the extent of clonality through field observations, therefore, a genetic approach was essential.

A combination of allozymes and RAPDs (random amplified polymorphic DNA) genetic markers revealed *Haloragodendron lucasii* to be extensively clonal, with the delineation of only seven genetic individuals (genets) across all four study sites. Furthermore, two of the four populations consisted of only a single genet. Thus, the species was found to be represented by far fewer individuals than previously suspected. In a population where only one genet was delineated, counts had previously suggested up to 40 individuals. More striking was the estimate for the largest population, where up to 700 individuals had been counted, but only three genets were delineated. These genetic results also imply an extremely low level of sexual reproduction. This is supported by low pollen viability and a lack of any seed set within the species.

The recognition of extensive clonality in *H. lucasii* provides a number of conservation implications (Fig. 3*a*). First, because there are so few genets in this species, and each are localised within a single population, it is vital to protect and preserve all populations, as the loss of any single population would lead to a substantial reduction in the overall genetic diversity remaining within the species. Second, it is clear that the collection of material for *ex situ* programs has to be considered differently to that of sexually reproducing species. Due to the localisation of genets, all populations would have to be sampled to obtain a representative *ex situ* collection, while extensive within population sampling is unnecessary. Finally, the location of new populations is essential as it may lead to the discovery of new genets, some of which may be capable of sexual reproduction.

While generating a series of conservation implications, not all of these implications have led to a change in management (management outcomes). For example, the first implication, that all populations of *H. lucasii* should be preserved, merely reinforced the suitability of current management practices. All populations of *H. lucasii* are already adequately reserved, thus, this conservation implication did not lead to a management outcome.

The second implication, that it is necessary to sample all populations in order to obtain an adequate *ex situ* collection has not yet lead to a management outcome, since the establishment and maintenance of an *ex situ* collection is not currently considered a management priority for *H. lucasii*. However, if the establishment of an *ex situ* collection becomes a management priority in the future, then this implication will have a practical management outcome.

The third implication, that the location of additional populations is necessary, has already led to an important management outcome: the establishment of the *Haloragodendron lucasii* Rediscovery Team. The *Haloragodendron lucasii* Rediscovery Team was established as a joint initiative of the New South Wales National Parks and Wildlife Service, the Ku-ring-gai Council, the Australian National University and local volunteer groups (Sydes *et al.* 1996). Volunteers from the team have been searching nearby areas for new populations of *H. lucasii*. This management outcome has already resulted in a practical outcome: the location of five additional populations.

Zieria prostrata (Hogbin and Peakall 1999)

Zieria prostrata (Rutaceae) is a geographically restricted endangered shrub, known from only four coastal headlands within a 3-km stretch of coastline near Coffs Harbour in northern NSW, Australia. The species was also presumed to have previously occurred at a disjunct fifth headland, 24 km south of its present distribution (Griffith 1992). As part of the recovery plan for this species (Griffith 1992), a reintroduction program was implemented in 1993, whereby mature plants were reintroduced into the apparently extinct site. Stock used in the reintroduction was cultivated from cuttings from an individual plant, apparently sampled from the site prior to extinction. Before the reintroduction program was continued, a genetic study was conducted to address questions that would assist in the development and implementation of a full-scale reintroduction program for the apparently extinct site. The following questions were addressed: (1) From a genetic perspective, does this highly endemic species consist of a single interacting population or a series of genetically isolated populations? (2) How does the genotype of an individual plant reputedly rescued from the now extinct population compare with individuals from the extant populations?

Random amplified polymorphic DNA (RAPD) analysis revealed an unexpectedly high level of divergence among the extant populations, implying little interaction among populations. Additionally, the individual plant sampled apparently from the extinct site prior to extinction was found to be closely related to individuals from one of the extant populations.

The extensive genetic divergence detected among *Z. prostrata* populations has a number of implications for the conserva-

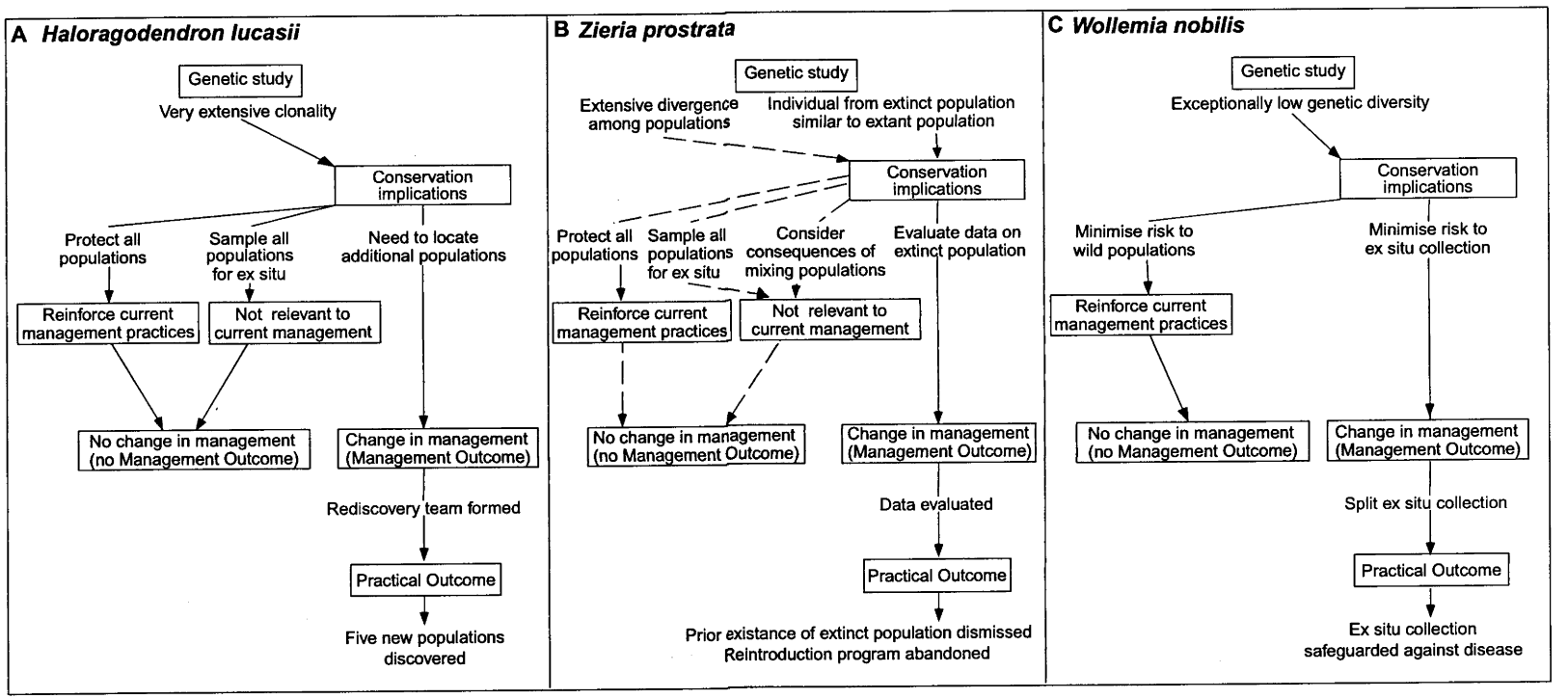

Fig. 3. A schematic representation of the conservation implications and practical outcomes of genetic study of the three plant species; *Haloragodendron lucasii*, *Zieria prostrata* and *Wollemia nobilis*.

tion management of the species (Fig. 3*b*). First, the loss of any one population would lead to a dramatic loss of genetic variation and thus it is important that all populations are conserved. Second, an adequate *ex situ* collection must sample the full range of genetic diversity from all populations. Finally, the potential genetic consequences of mixing populations may also be an important conservation consideration if further translocations are to proceed. Furthermore, the genetic similarity of the individual apparently from the extinct population with individuals from one of the extant population raised doubts about the population's existence and thus led to the recommendation that data on the extinct population needed to be evaluated. Given that extensive genetic divergence occurs among populations within this species across a geographic range of only 3 km, it was expected that a population 24 km south would show considerable divergence from the other populations. This was not the case.

As for *Haloragodendron lucasii*, while a series of conservation implications have been provided not all have led to management outcomes. The conservation implication that all populations of *Z. prostrata* should be preserved, may at first seem to be an important implication. However, given that only four populations are known, all populations require preservation irrespective of the genetic considerations. Indeed, all four populations were gazetted as nature reserve in 1995, before completion of this study. Thus, the implication, while reinforcing the importance of protecting all populations, did not result in a change of management.

The conservation implications concerning the adequacy of the *ex situ* collection and the consequences of mixing populations, have not yet led to management outcomes. Neither an *ex situ* collection or translocation are currently considered management priorities. Instead, a number of more cost-effective and lower-risk alternatives are being considered for enhancing the extant populations, including exclusion of seed predators to enhance production of viable seed, and artificial soil disturbance to enhance germination.

In contrast to the previous implications, the unexpected genetic similarity of the sample reputedly taken from the now extinct population, has already had a management outcome. The finding led to a thorough investigation of the evidence supporting the prior existence of the population. Unexpectedly, all avenues of investigation failed to provide any convincing evidence that *Z. prostrata* grew at the site. This lead to the conclusion that the prior existence of the extinct population was doubtful and forced the abandonment of a major reintroduction program. This represents a major change in the management plan for the species, with a clear practical outcome. Without this genetic study, further expensive and time-consuming reintroduction would have proceeded unnecessarily. Funds have now been redirected to the management of the extant populations.

Wollemia nobilis (Peakall, unpubl. data)

The discovery of the Wollemi pine, *Wollemia nobilis* (Araucariaceae), in December 1994 made international headlines. The discovery of the relictual conifer, previously known only from the fossil record and thought to have been extinct since the Cretaceous, has been regarded by some as the 'botanical discovery of the century' (Jones *et al.* 1995). Presently, the Wollemi pine is known only from two small populations in the Wollemi National Park, about 200 km west of Sydney, NSW, Australia. Recognising the botanical significance of the species, the Royal Botanic Gardens of Sydney (RBGS) have begun a program with the ambitious aim of propagating the species on a commercial scale. By making plant material widely available to the public, it is hoped that the risk of illegal and potentially devastating collection of the species in the wild will be removed.

While the Wollemi pine produces seed annually, and both seedlings and juveniles are apparent within the populations, the coppicing habit of the species indicates that it may be extensively clonal. An understanding of the extent of clonality in the Wollemi pine is crucial for the design of an *ex situ* collection that captures the full range of genetic diversity, yet avoids unnecessary replication of clones. Understanding the extent of clonality may also provide insight into the rates of recruitment and the possible age of the clones in the wild. For these reasons, a genetic survey was conducted to investigate genetic variability and the extent of clonality within and among the two populations, on the basis of samples within the *ex situ* collection.

As apparent in the case of *Haloragodendron lucasii*, genetic investigation of potentially clonal species can produce unexpected results that are not obvious without genetic study. In the case of the Wollemi pine, an extensive genetic survey of 13 allozyme loci and more than 800 AFLP loci, failed to detect any genetic variation. Given the scale of the genetic survey, which included adults from both sites and progeny from one site, the results indicate unusually low levels of genetic diversity within the species. Few, if any, other plant species are known to exhibit such low levels of genetic diversity.

The complete lack of genetic variation in the Wollemi pine has hampered any attempt to assess the extent of clonality in the species. Furthermore, although genetic theory predicts that a combination of factors, including clonality, genetic drift and inbreeding, can contribute to the loss of genetic variability, we are presently unable to determine the relative contributions of these different factors. Ongoing research is now focussed on assessing the levels of genetic variability in other species in the family. This will provide crucial baseline data for interpreting the findings in the Wollemi pine.

The finding of exceptionally low genetic diversity in the Wollemi pine increases concern about the species vulnerability to exotic pathogens, leading to a conservation implication that strategies to minimise the risk of exotic pathogen infection are crucial for both the wild and *ex situ* populations (Fig. 3*c*). With respect to the wild populations, while reinforcing current management practices, this conservation implication has not led to a change in management, since a restricted access and

hygiene policy was already in place. In contrast, concern about the vulnerability of the *ex situ* collection to a pathogen epidemic has accelerated the process of splitting up the collection among multiple facilities. The consequent reduction in the risk of a disease epidemic destroying the entire *ex situ* collection represents a tangible practical outcome.

Lessons from our genetic studies

Our genetic studies each provided a number of conservation implications for the species concerned; however, not all of the implications actually led to practical outcomes. There were three main reasons why the conservation implications did not translate to management outcomes. First, similar recommendations were already in place. Second, management recommendations, while sound, were not relevant to the current management priorities. Third, irrespective of the genetic outcome, a given recommendation prevails as the preferred management option. Thus, the management in place, and the management planned for the near future, strongly influenced the outcomes. We explore these points further in the following discussion.

In the case of both *Zieria prostrata* and *Haloragodendron lucasii*, the extensive divergence among populations led to the management recommendation that all populations should be conserved. While a common recommendation from genetic studies, concerns about conserving genetic diversity become relevant only when all populations are not already protected or when it is not possible to conserve all extant populations adequately. This highlights a critical point that is frequently overlooked. The best way to conserve genetic diversity is to preserve all populations, in which case we do not need formal genetic studies. Indeed, for many endangered plants, the number of populations is already so low that we cannot afford to loose a single population (Peakall and Sydes 1996). Thus, ecological considerations lead to the same recommendation as genetic considerations. If all populations are protected, or if it is possible to protect all populations, genetic study may not be justified, since by preserving all populations the species total genetic variability is automatically conserved.

If it is not possible to reserve all populations, and a choice exists as to which populations can be reserved, genetic research may provide valuable insights for reserve design (e.g. Prober and Brown 1994; Richter *et al.* 1994; McCue *et al.* 1996; Ledig *et al.* 1997; Palacios and Gonzales-Candelas 1997). However, for genetic knowledge to actually contribute in a practical way to reserve design, procedures must be in place to allow genetic results to be incorporated into the criteria for reserve selection. We are not aware of any study that has actually (rather than potentially) contributed to reserve design in this way. If such cases do exist, it will be of interest to determine if the genetic data actually influence the reserve design. We suspect that more often, than not, genetic considerations will merely reinforce the preferred design. For example, ecological considerations will frequently suggest that the full range of the species should be represented in the reserve system. If this is achieved, this may adequately capture the genetic diversity without the need for formal genetic study.

The extensive genetic divergence detected among populations of both *H. lucasii* and *Z. prostrata* led to management recommendations for obtaining a representative *ex situ* collection that would conserve the species total genetic diversity. However, in both species, an *ex situ* collection is not considered a management priority. Thus, this implication has not yet translated into a practical outcome in either species. In *H. lucasii*, without formal genetic study the extent of clonality would not be known and unnecessary sampling and over representation of genets would undoubtedly occur. This suggests that if clonality is possible, and an *ex situ* collection is justified, genetic study may be critical for achieving a representative collection (Peakall and Sydes 1996; Sydes and Peakall 1998). If it is feasible to sample all populations, and there are no indications of clonality or other non-random genetic structure, then effective sampling of genetic diversity may be achieved by random sampling of each population. These assumptions are made in the various guidelines on *ex situ* sampling (e.g. Brown and Briggs 1991; BGCI 1995; ANPC 1997), and may be sufficient to achieve a representative collection in *Z. prostrata* without further genetic analysis.

The conservation implication in *Z. prostrata*, that the potential genetic consequences of mixing populations should be a consideration if translocations proceed, provides an example of a management recommendation that should be made, regardless of the genetic outcome. Irrespective of genetic results, or even without formal genetic study, any plan to mix populations raises some important and unresolved issues. On one hand, even in the absence of detectable divergence, there may be population differentiation in other traits, particularly quantitative traits that may be more closely linked to fitness (Storfer 1996). In which case, irrespective of genetic results, the best management recommendation will be to retain, if possible, population distinctiveness. On the other hand, although potentially breaking up coadapted gene complexes, the mixing of populations offers the benefit of increasing genetic diversity and avoidance of inbreeding (Havens 1998). Given these conflicting possibilities, Havens (1998) has suggested a dual translocation strategy in which reintroductions maintain population distinctiveness, while introductions ensure genetic variation by mixing population sources.

The Wollemi pine example also illustrates a case where a single management recommendation would be adopted, regardless of the genetic outcome. Concerns about human impact on the populations and the potential of introducing exotic pathogens, has led to strict controls on access to the Wollemi pine in the wild. This management strategy remains crucial regardless of the underlying patterns of genetic diversity. While it is true that the findings of low genetic diversity

reinforce the existing management, either way the outcome will not change the management strategy already in place. In a similar way, while the findings of low genetic diversity did provide the impetus for splitting up the *ex situ* collection, so as to spread the risk of a pathogen epidemic, this strategy should perhaps have already been in place to minimise a range of potential disasters, both biological and non-biological.

Future directions

As evident in this volume, Australia has been and continues to be a major contributor to the population genetic study of plants, including many rare species. For many of these studies, rarity *per se* appears to have been the justification for conducting the study, with the hope that the results will lead to conservation outcomes. However, while these genetic studies have and will continue to reveal much about the biology of the species in question, few have demonstrated practical conservation outcomes.

We suggest that future genetic research on rare plants should proceed on two separate fronts. On one front, we must continue the large scale, integrated ecological and genetic studies of 'model systems'. These studies need not be motivated by the potential for a direct practical outcome to conservation, but instead are motivated by the need to experimentally tackle the many outstanding questions in conservation and population genetics. The exploration of phylogeographic patterns can also provide important conservation related insights (see Moritz 1994), yet the application of molecular ecology for exploring phylogeography in plants lags well behind that in animals and will clearly be a profitable area of future research. On a separate front are those genetic studies that are motivated by the desire for practical outcomes. Frequently, such studies will be conducted on a slim budget within the context of a particular recovery program, and with the reasonable expectation of a practical outcome. Such studies are best focussed on a specific hypothesis. Clearly appropriate hypotheses can be formulated only when basic knowledge of the natural history and biology of the species is available. The gathering of such knowledge must precede genetic study, and will be crucial for identifying whether there is potential for genetics to contribute in a practical way. For example, if field observations suggest that clonality is likely, it may then be appropriate to address the question 'Is the species clonal?'. However, before proceeding with genetic study, the management context also needs to be considered. This may be done by considering a range of possible outcomes, and then determining how these would translate into conservation implications, and whether or not these in turn will translate into management outcomes. If none of the possible outcomes leads to a potential change in management, genetic research for a practical outcome may not be justified. However, if any one of the possible, but as yet unknown, outcomes will produce a change in management, genetic research should proceed. Consideration of the priorities for the genetic study of rare plants proposed by Peakall and Sydes (1996) combined with our generic flow diagram (Fig. 2), may assist in this decision making process. We believe that following this simple process of evaluating the prospects of a practical outcome will ensure that conservation agencies will get the best value for money from the genetic studies of rare plants.

Acknowledgments

We are grateful to the Australian National University, Sydney Royal Botanic Gardens, and the National Parks and Wildlife Service of NSW for providing funding for the projects described here. Marita Sydes and Patricia Hogbin were both supported by Australian Postgraduate Award (Industry) PhD scholarships.

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Manuscript received 20 November 1998, accepted 29 May 1999

Appendix. Source journals for the evaluation of the last 10 years of genetic research on rare plants

American Journal of Botany, Australian Journal of Botany, Biodiversity and Conservation, Biological Conservation, Biological Journal of the Linnean Society, Canadian Journal of Botany, Conservation Biology, Evolution, Heredity, International Journal of Plant Sciences, Journal of Heredity, Molecular Ecology, Plant Systematics and Evolution, Restoration Ecology, Systematic Botany.

The effective management of threatened flora: lessons from the case of *Zieria 'prostrata'* ms

PATRICIA M. HOGBIN[1] and ROD PEAKALL[1]

The endangered plant *Zieria 'prostrata'* ms is known from only four headlands along three kilometres of the north coast of New South Wales, Australia. Given its restricted range and small population sizes, *Zieria 'prostrata'* has been the subject of extensive management and research for almost a decade. In this paper, we review the history of management and research actions undertaken on *Z. prostrata* and evaluate their practical outcomes. By revisiting past management and research actions, and assessing their outcomes, we can learn much about the effective management and recovery of threatened flora. This review highlights five valuable lessons. First, effective survey is a priority for many rare plants. Second, accurate information and documentation are essential for effective conservation. Third, critical evaluation of the need for *ex-situ* conservation is necessary. Fourth, critical evaluation of the need for population enhancement and the most effective methods by which this can be achieved is a high priority. Fifth, adequate guidance of all those involved in recovery implementation is vital.

Key words: Threatened flora, Conservation, Management, Recovery plan, *Ex-situ*, Translocation, Population genetics.

INTRODUCTION

THE task facing flora conservation managers within Australia is difficult. For example, within New South Wales alone, 39 plant species have become extinct and over 200 may face extinction if no action is taken to reverse their decline (Schedule 1, New South Wales *Threatened Species Conservation Act 1995*). The limited resources available to conservation managers increases the difficulty of conserving such a vast number of threatened species. It is therefore vital that cost efficient and effective recovery programmes are developed and implemented.

To achieve effective recovery of threatened plant species, actions that are most likely to have a positive influence on the survival of a species need to be identified. One way of achieving this goal is to examine past conservation efforts. In this paper, we review the history of research and management undertaken on the endangered plant *Zieria 'prostrata'*. We then evaluate the practical outcomes of such actions and identify lessons for the management and recovery of threatened flora in general.

REVISION OF PAST MANAGEMENT AND RESEARCH

At the start of our involvement in the recovery of *Zieria 'prostrata'* in 1996, we attempted to review all available historical information on the management of the species. This included the collection of available written documentation held by the NSW National Parks and Wildlife Service (NSW NPWS), personal communication with past and present members of the Recovery Team and a written survey to a range of people involved in the management of *Z. prostrata*.

The discovery of *Zieria 'prostrata'*

In 1981, a putative new species of *Zieria* was found on the north coast of New South Wales near Coffs Harbour. This taxon was subsequently given the manuscript name *Zieria 'prostrata'* (Armstrong, pers. comm.). Like many *Zieria* species, although widely regarded as a distinct species, a description of this taxon has not been formally published. The taxon is referred to as *Zieria* species Q in the Flora of New South Wales (Armstrong 1991) and is listed as an endangered species under the name *Zieria 'prostrata'* at both a Sate (*Threatened Species Conservation Act 1995*) and National (*Endangered Species Protection Act 1992*) level.

Zieria 'prostrata' was initially known from only three headlands along a three kilometre stretch of coastline (Fig. 1). The total population size was then estimated to be approximately 400 individuals (Griffith 1992). A fourth population of *Z. prostrata*, comprised of only nine individuals, was subsequently found at Diggers Point in 1994.

Initial recovery plan

Concern was expressed for the survival of *Zieria 'prostrata'* due to its small population size and restricted geographic range. Furthermore, the species was presumed to have previously occurred on a fifth headland, Bonville Headland, 24 kilometres south of its extant distribution (Griffith 1992) (Fig. 1). Consequently, a recovery plan (Griffith 1992) was prepared under the then Australian Nature Conservation Agency (ANCA) Endangered Species Programme.

Little was known of the biology of *Z. prostrata* at the time of writing the recovery plan.

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PACIFIC CONSERVATION BIOLOGY Vol. 6: 238–44. Surrey Beatty & Sons, Sydney. 2000.

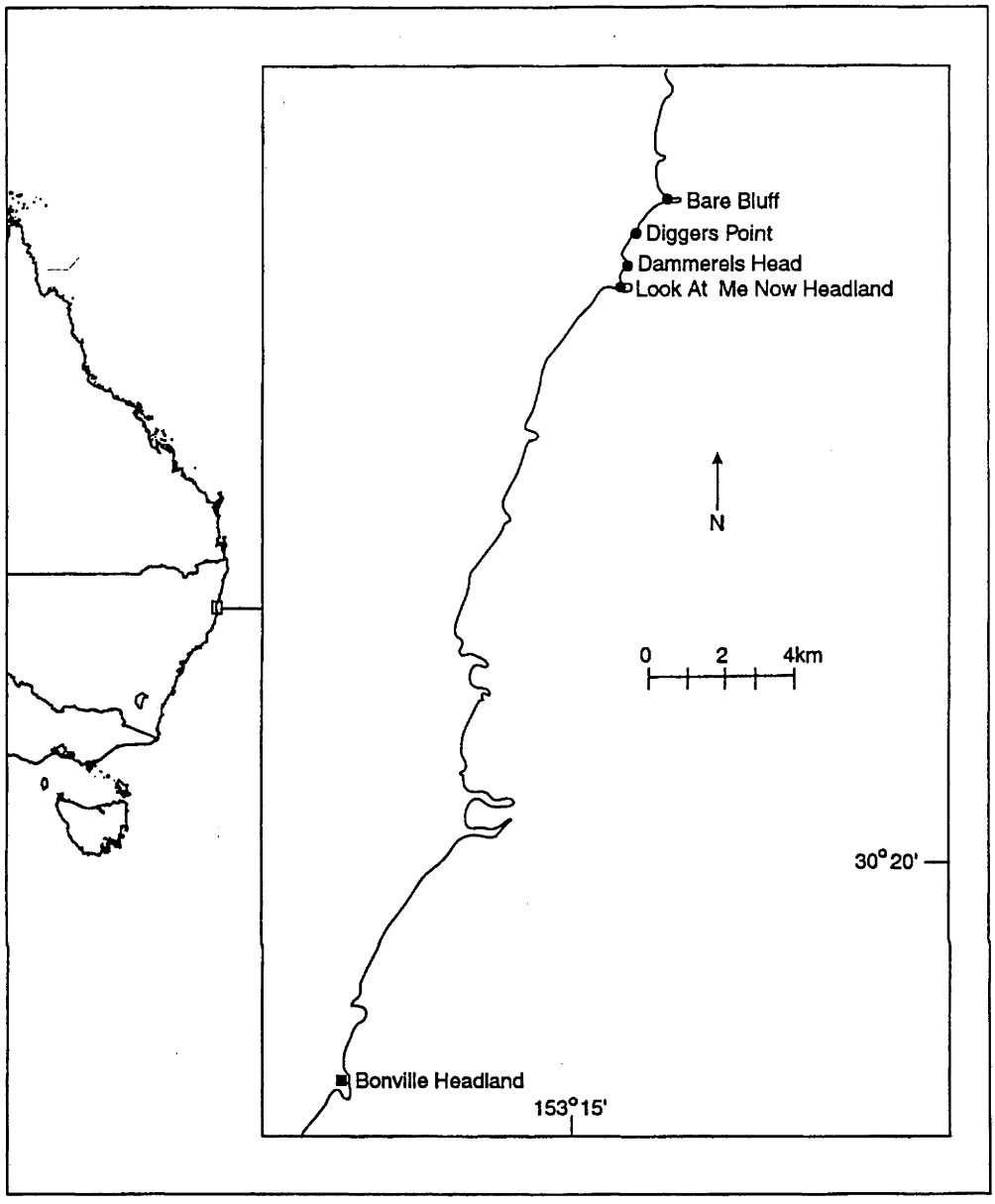

Fig. 1. Distribution of *Zieria 'prostrata'* showing the location of the extant populations (•) and the site where the previous occurrence of *Z. prostrata* was dismissed after further investigation (■).

Armstrong (pers. comm.) found the anthers of *Z. prostrata* to be devoid of pollen and concluded that the species was male sterile. However, a local naturalist observed large seed crops (Griffith 1992). A three day field survey found no evidence of seedlings or juveniles, giving the impression that populations were dominated by older individuals (Griffith 1992).

The recovery plan recognized extensive habitat degradation caused by uncontrolled vehicle access, pedestrian activity, and weed invasion as the main threats to the survival of the species. The priority management actions centred on translocation, habitat protection, restoration and reservation (Table 1). The priority research tasks included a diverse range of activities: ecological research, genetic research and the establishment of a genetically representative *ex-situ* collection.

Ex-situ collection

An *ex-situ* collection of *Z. prostrata* was established in 1992 "as a safeguard against possible future losses from the wild" (Griffith 1992). Unfortunately, much of the process was poorly documented. It is known that cuttings were collected from two or three of the extant populations (Dammerels Head, Look At Me

Table 1. Priority management actions and research tasks proposed in the initial *Zieria 'prostrata'* recovery plan (Griffith 1992).

Priority management actions	Priority research tasks
• Translocation (enhance smallest extant population and reintroduce plants to site of extinct population)	• Monitor population levels
• Minimize or arrest habitat degradation	• Study population dynamics (e.g., seed biology, fire ecology)
• Habitat restoration and weed control	• Assess patterns of genetic diversity in *ex-situ* collection and natural populations
• Reserve habitat	• Establish genetically representative *ex-situ* collection
• Fire prevention	
• Install interpretive signs	

Now Headland and possibly Bare Bluff) and also from an individual apparently rescued from the now extinct Bonville Headland population. These cuttings were then propagated and maintained by the Coffs Harbour City Council. It is not known how many plants were sampled, nor from which plants the cuttings were taken. No record of the origin and success of individual cuttings was maintained.

Translocation

Within one year of establishing the *ex-situ* collection, it was used as a source of stock for a translocation programme. Plants were reintroduced to Bonville Headland and population enhancement occurred at two of the extant populations (Look At Me Now Headland and Dammerels Head) (S. Clemesha, pers. comm.). Translocated plants were not individually tagged and no map of the translocation was made, nor are the numbers of plants involved known. The success of the translocated plants was not monitored.

Habitat protection

In 1994 ongoing habitat protection measures were implemented. Vehicular access to the headlands was restricted. A weed control programme commenced which focused in particular on eradicating *Chrysanthemoides monilifera* ssp. *rotundata* (Bitou Bush), *Lantana camara* (Lantana) and *Pennisetum clandestinum* (Kikuyu) in the vicinity of *Zieria 'prostrata'* plants.

Land reservation

In 1995 all four known populations of *Z. prostrata* were included within the Moonee Beach Nature Reserve. This achievement was largely a result of submissions from the local Ulitarra Conservation Society (S. Clemesha, pers. comm.), political interest, and strong local support for the reservation of the headlands. This strong interest was not solely due to the presence of *Z. prostrata*, and can also be contributed to a proposal for a sewerage outfall off Look At Me Now Headland. The responsibility for management of the headlands was thus passed from the Coffs Harbour City Council to the NSW NPWS.

Genetic research

Population genetic research was conducted on *Z. prostrata* in 1996 (Hogbin and Peakall 1999; Hogbin *et al.* 2000). The NSW NPWS intended using this genetic insight to assist in the design of a second, and larger reintroduction programme for Bonville Headland. Despite their close proximity, all four populations of *Z. prostrata* were significantly genetically differentiated (Hogbin and Peakall 1999). By contrast, the plant apparently rescued from Bonville Headland prior to the extinction of the population, was genetically similar to an individual from Dammerels Head. This unexpected similarity was initially attributed to a labelling error during the *ex-situ* phase. Subsequent genetic research ruled out a labelling error as the "source" plant growing in the botanic gardens and a reintroduced individual showed the same genotype.

Prompted by this anomaly, details of the extinct population were re-investigated. Searches for herbarium and cultivation records reputedly taken prior to the extinction of the population were made, and those people who were associated with the original collections were interviewed. All avenues of investigation failed to provide any convincing evidence that *Z. prostrata* grew at Bonville Headland. The available evidence suggests the collection instead most likely came from Dammerels Head, but was incorrectly ascribed to Bonville Headland. Presumably, subsequent searches at Bonville Headland failed to find any trace of the plant, leading to the claim of extinction, which in turn provided motivation for an entire reintroduction programme. In light of these findings, the Recovery Team concluded the prior existence of *Z. prostrata* at Bonville Headland was doubtful and abandoned the reintroduction programme (NPWS 1998).

Evaluation of *ex-situ* collection and translocation programme

In 1996 the *Z. prostrata* Recovery Team evaluated the status of the *ex-situ* collection and the success of the 1993 translocation programme. The *ex-situ* collection was found to be in poor condition, comprising unhealthy, root bound plants held in a non-sterile corner of the Coffs

Harbour City Council depot. The collection was also considered to inadequately fulfil its objective of being genetically representative, given that only two or three of the four populations were represented and no information on the number of genetic individuals was available. Given the poor state of the collection and the secure reservation of all known populations, the *ex-situ* cultivation programme was abandoned.

Evaluation of the translocation programme was hampered by poor documentation. It was impossible to determine how many plants were reintroduced, or where they were planted. By interviewing individuals involved in the initial programme at the site itself, we estimated that Look At Me Now Headland and Dammerels Head were enhanced with 150 and 50 individuals respectively. At most, 19 (12.7%) of the translocated plants survived at Look At Me Now Headland and only 5 (10%) at Dammerels Head. It is not known how many plants were reintroduced to Bonville Headland, with only one surviving plant relocated.

Investigation of reproductive ecology

A study of the reproductive ecology of the species commenced in 1996 in order to investigate population stability and possible reproductive limits. Contrary to a previous observation, pollen was found to be viable. Bagging experiments revealed that the species is capable of pollination in the absence of pollinators (autogamy). Seed production was found to be substantial, however seed predation by an unidentified wasp larvae was apparent at two of the four populations. The seed of *Z. prostrata* were found to germinate readily and seedlings were observed *in-situ*. Collection of soil samples over a 12 month period revealed the species maintains a short-lived transient seed bank. A seed burial experiment revealed that shallow burial and soil disturbance may enhance seed germination. Subsequent research revealed that small scale soil disturbances increase seedling recruitment (P. M. Hogbin, unpubl. data).

In the absence of major limits to reproduction, current land protection and land management may be sufficient to ensure population stability. If in the future, however, additional management is required to increase recruitment and population stability, this research revealed a number of simple and cost effective management options worthy of further investigation, including:

(i) soil disturbance to enhance germination,

(ii) exclusion of seed predators to enhance viable seed production, and

(iii) seed collection and artificial dispersal to extend population size.

Population size re-evaluated

Extensive survey of the extant populations was conducted in 1996, and it became apparent that population sizes had been underestimated in the initial recovery plan. For example, Look At Me Now Headland, initially thought to support only 25 plants (Griffith 1992), contained more than 130 mature plants. The total population size is now considered to be over 1 000 individuals (NPWS 1998), more than double the 400 estimated in the initial 1994 recovery plan (Griffith 1992).

Similar taxon discovered

Searches for *Z. prostrata* over 1996–1999 along 600 km of the New South Wales coastline located eleven headlands between Port Stephens and Byron Bay which support a taxon possessing similar morphology and growth habit to *Zieria 'prostrata'*, hereafter referred to as *Zieria* sp. aff. *smithii* The relationship between *Z. prostrata* and *Z.* sp. aff. *smithii* was unclear and research into the taxonomy of this group was considered a high priority given the management implications. For example, if *Z.* sp. aff. *smithii* was *Z. prostrata*, an expansion of the recovery plan to cover the management needs of the additional populations would be required. On the other hand, if *Z.* sp. aff *smithii* was found to be a distinct species, its taxonomic and conservation status would need to be urgently assessed.

Second recovery plan published

A second recovery plan for *Z. prostrata* was published in 1998 (NPWS 1998). In the light of the afore mentioned management actions and better understanding of the biology and status of the species, a set of new priorities emerged (Table 2). Priority actions centred upon further survey, taxonomic research, and continued biological research and management of threatening processes. Neither translocation or *ex-situ* conservation were considered priority management options because land protection and land management appeared sufficient to ensure population stability, at least in the short term.

Table 2. Priority actions proposed by the second *Zieria 'prostrata'* recovery plan (NPWS 1998).

Priority Actions
• Survey: Identify, reserve and protect all *Z. prostrata* populations.
• Resolve taxonomy and distribution of *Z.* sp. aff. *smithii*.
• Continue biological research to enable informed management.
• Continue to eliminate or minimize threatening processes (e.g., weeds, vehicles and pedestrians).
• Evaluate the need for population enhancement and options for protection against catastrophe.
• Inform the community about *Z. prostrata* and its habitat.

LESSONS FROM THE CASE OF *ZIERIA 'PROSTRATA'*

Notwithstanding problems along the way, a decade or so after recovery efforts commenced, *Z. prostrata* appears to have a secure future. The species is now protected within a reserve network, habitat protection measures have minimized disturbance, and the populations appear stable. Knowledge of the biology of the species has also been gained from a range of research that will assist in the effective management of the species into the future.

Our review of the *Z. prostrata* recovery process revealed that some actions improved the survival chances of the species, whereas others were of limited value. If the conservation of threatened flora is to be effective it is vital that we learn from past mistakes and build on successful strategies. Here we highlight five lessons revealed by the case of *Z. prostrata* which can hopefully be applied to the effective management of other threatened plants.

Effective survey is a high priority

The case of *Z. prostrata* highlights the need for effective and extensive survey. For example, additional within-population surveys revealed that population size was considerably larger than initially believed. Surveys of nearby headlands also located an additional population of *Z. prostrata*. While this fourth population at Diggers Point supports only nine individuals, it may be of considerable conservation value as it is highly genetically distinct from the remaining three populations despite it being geographically intermediate between two of the populations (Hogbin and Peakall 1999).

Surveys along some 600 km of coastline also identified 11 previously unknown populations of the morphologically similar headland taxon Z. sp. aff *smithii*. The initial recovery plan (Griffith 1992) stated that "a search in 1988 for Z. *prostrata* on other headlands along some 100 km of coastline to the north and south of Coffs Harbour proved unsuccessful" and claimed that "Z. *prostrata* is unlikely to occur at hithero unknown locations." This 1988 survey, however, did not extend far enough (A. G. Floyd, pers. comm.) to include the range of Z. sp. aff *smithii*. While ongoing taxonomic research suggests Z. sp. aff *smithii* is distinct from *Z. prostrata*, had this not been the case, the discovery of these additional eleven populations would have had profound implications upon both the conservation status and management of *Zieria 'prostrata'*.

The impact of further survey on the conservation status of threatened flora can be illustrated by some recent changes to the Schedules of the New South Wales *Threatened Species Conservation Act 1995*. For example, the New South Wales Scientific Committee recently downlisted a number of species from Endangered to Vulnerable based upon further surveys that revealed they were more abundant than initially believed (e.g., *Velleia perfoliata*, *Kunzea rupestris* and *Grevillea molyneuxii*). Another species, *Pultenaea campbellii* was removed from the Schedules after further survey. More effective survey may also reveal that a species is rarer than initially believed. For example *Persoonia bargoensis* and *Acacia bynoeana* were recently upgraded from Vulnerable to Endangered and *Persoonia hirsuta* from Rare to Endangered (T. Auld, pers. comm.).

Accurate information and documentation are essential for effective conservation

The apparently incorrect assumption that *Z. prostrata* once occurred at Bonville Headland provides an example of how a lack of careful documentation and validation can ultimately and unintentionally lead to misinformation. Without the genetic research and the subsequent search for information supporting the prior existence of the population, further expensive and time consuming reintroduction may have proceeded unnecessarily.

The lack of information on the establishment and maintenance of the *ex-situ* collection subsequently contributed to its abandonment. This failure to document the process occurred despite Griffith (1992) highlighting the importance of such records in the initial recovery plan: "Of course, accurate records of the collection, propagation and cultivation histories of living collections would need to be made".

Failure to individually tag and map the translocated plants prevented evaluation of the translocation programmes success. Again, this mistake occurred despite Griffith (1992) emphasising the importance of such documentation in the initial recovery plan: "As with the proposed *ex-situ* cultivation of *Z. prostrata*, it is imperative that detailed records of *in-situ* cultivation be made".

As best we can assess, the lack of documentation appeared to occur at multiple levels, involving multiple individuals and multiple agencies. A lack of documentation or the inability to access information appears to be a common problem in threatened species recovery and is complicated by the diverse range of organisations and individuals involved in recovery efforts. More often than not, a great deal of information is unavailable and stored in the heads or personal files of volunteers, contractors or conservation agency staff.

While government conservation agencies do have policy on appropriate storage of information, it still appears much vital information remains

with volunteers or contractors. It is therefore crucial that recovery programmes define the degree and extent of reporting required and establish clear rules on who is responsible and where records should be maintained. The electronic age makes data sharing more feasible than ever before, but also brings new responsibilities to ensure accessibility and physical integrity of the data. We suggest that the National or State conservation agency responsible for recovery implementation be responsible for providing adequate training in documentation to the many volunteers, contractors and staff involved in the recovery process, and ultimately take responsibility for the secure housing of documentation.

Critical evaluation of the need for *ex-situ* conservation is needed

The initial recovery plan for *Z. prostrata* recommended the establishment of an *ex-situ* collection of *Z. prostrata* "as a safeguard against possible future losses from the wild" (Griffith 1992). However, while such losses may occur, particularly when a proposed development will destroy a population or when the reservation of a highly disturbed population is not possible, in this case such losses seemed unlikely. Instead, effective conservation of this species may best be achieved by managing the natural populations. Such efforts, if possible, are far more effective than *ex-situ* conservation which requires considerable effort and expense to initiate and maintain. In the case of *Z. prostrata*, an expensive and ineffective *ex-situ* collection with little real conservation value was discarded only four years after its establishment.

Critical evaluation of the need for population enhancement and the most effective methods by which this can be achieved is a high priority

Population enhancement is frequently proposed for endangered species before causes of the species decline are identified, likely threatening processes identified and minimized, or detailed investigation of the biology of the species have been made. In some instances, such as for *Z. prostrata*, biological investigation may suggest that population enhancement is not necessary. Many populations of threatened plant species may not require any form of active management for numbers to remain stable or may increase in size once threatening processes have been removed or lessened.

The initial recovery efforts for *Z. prostrata* concentrated on population enhancement through translocation, however, the reason for translocation was not obvious. The only justification for the translocation programme was: "Look At Me Now Headland has suffered considerable disturbance as a result of indiscriminate vehicle usage . . . it is probable that *Z. prostrata* has here experienced losses. Given that the remaining population at Look At Me Now Headland is extremely small, the strategy of *in-situ* cultivation seems appropriate" (Griffith 1992). In 1996, however, more extensive survey revealed that the population was at least five times larger than initially believed. Translocation was implemented at this headland in 1993 before land tenure was secured, land management was implemented and apparently even before a detailed site survey was conducted. At the same time, translocation occurred at Dammerels Head, a relatively large and undisturbed population, even though this task had not been proposed in the recovery plan.

If, after critical evaluation, population enhancement is necessary, it is vital to then determine the most effective method to achieve this goal. There are numerous active management options available for the population enhancement of endangered plants. Active management involves the use of manipulative techniques to enhance population size by directly influencing the survival of individual plants or a population. At the same time these manipulative techniques have the potential to alter the genetic structure of a population and thus its evolutionary development. Consequently, it is important that such techniques be used in a management context only when necessary for the long-term survival of a species and that they are based upon sound scientific knowledge.

Active management options can be ranked according to level of manipulation and thus the level of potential risk to the species. It is desirable to consider the feasibility of the less evolutionary disruptive techniques first and then move on to those "higher risk" options if required. At the lower risk end of active management there are techniques which manipulate or restore natural processes with the aim of increasing recruitment. Such techniques may include hand pollination, burning, clearing, soil disturbance, or removal of grazing animals. The advantage of such techniques is that they are cost effective, cause the least evolutionary disruption, and are more likely to stabilize a population at its natural density. At the high risk end of active management options we have translocation. Translocation, with its associated high maintenance and high cost may best be viewed as a last resort when all other options have failed. Additionally, to date, the majority of translocation programmes have generally been unsuccessful (ANPC 1997).

The case of *Z. prostrata* highlights how a relatively simple investigation of the reproductive ecology of a species can identify appropriate active management options. In this case, cost effective and lower risk alternatives to translocation were identified, including exclusion of seed predators to enhance viable seed production and soil disturbance to enhance germination. If population enhancement proves to be necessary in the future, these options should be investigated further and are favoured over translocation (NPWS 1998).

Adequate guidance of all those involved in recovery implementation is vital

It is important to emphasize that the successful management of *Zieria 'prostrata'* could not have been achieved without the co-operative efforts of numerous people from a diverse range of organizations and fields. The *Z. prostrata* recovery team, since its inception almost a decade ago, has comprised representatives from a diverse range of organizations including the NSW NPWS, Local council, the Australian National University, ANCA (now Environment Australia) and a local conservation society. Such a diverse membership is vital and the effective recovery of threatened species can only be achieved with such ongoing collaborations. The value of involving several organizations and a range of expertise in the recovery process is also illustrated by Myronivk (1995) and Smales *et al.* (1995).

It is vital, however, that all recovery team members receive adequate guidance and direction throughout the implementation process. In their evaluation of the recovery process in animals, Backhouse and Clark (1995) noted that successful implementation can be elusive in environments dominated by poor information and great uncertainty, and when appropriate specialization and assistance are lacking. This also seems to have been the case for *Zieria 'prostrata'*. The rapid response to the proposal for translocation of *Z. prostrata* can be largely attributed to the involvement of a group of enthusiastic volunteers. Their enthusiasm may have been further enhanced by the threat of an Ocean outfall at Look At Me Now Headland. Enthusiastic volunteers can clearly provide considerable assistance in the implementation of recovery plan actions, with the potential to achieve outcomes at accelerated rates. However, in the case, of *Z. prostrata*, this intense activity appeared to coincide with a period of limited guidance. Clearly, to achieve effective management, it is vital that the conservation agency responsible for recovery implementation provide adequate and ongoing guidance throughout the recovery process.

ACKNOWLEDGEMENTS

We would like to thank all people involved in the recovery of *Z. prostrata*, in particular the current members of the *Z. prostrata* recovery team; Tony Auld, Steve Clemesha and Andrew Steed. We would also like to thank all those individuals who provided information on the past management of *Zieria 'prostrata'*, including John Wrigley, Alex Floyd and in particular Steve Clemesha. We are also grateful to the editor and three anonymous reviewers whose comments greatly improved this manuscript. This review and much of the research work was completed while P. M. Hogbin was a recipient of an Australian Postgraduate Research Award (Industry) with additional funding provided by the New South Wales National Parks and Wildlife Service and the Australian National University.

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